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(54) Title: ANTAGONISTS OF HSP90/CDC37 AND METHODS OF USING THE SAME

(57) Abstract: This disclosure concerns compounds which are useful as HSP90/CDC37 antagonists and are thus useful for treating a variety of diseases and disorders that are mediated or sustained through the interaction of HSP90 and CDC37. This disclosure also relates to pharmaceutical compositions that include these compounds, methods of using these compounds in the treatment of various diseases and disorders, and processes for preparing these compounds.



ANTAGONISTS OF HSP90/CDC37 AND METHODS OF USING THE SAME

CROSS-REFERENCE

5 This application claims the benefit of U.S. Provisional Patent Application No. 62/022,008 filed July 8, 2014, which application is incorporated herein by reference in its entirety.

INTRODUCTION

10 Hepatocellular carcinoma (HCC), the most common adult liver malignancy, is the seventh most common cancer and the second most frequent cause of cancer-related death worldwide. Most of the burden (80%) of HCC is borne in the developing world, such as Eastern and Southeast Asia and sub-Saharan Africa, where the dominant risk factor is chronic infection with hepatitis B virus (HBV), together with exposure with aflatoxin B1. In developed countries, including North America, Europe, and Japan, the dominant risk factor is chronic infection with
15 hepatitis C virus.

 HCC has a poor prognosis, partly due to late diagnosis of the disease and lack of effective therapeutic options. Most patients remain asymptomatic until the disease is advanced. Although more than 50 drugs that target different biomarkers or signaling pathways are in clinical trials for HCC treatment, as yet there is no therapeutic agent superior to sorafenib, a
20 tyrosine kinase inhibitor, which was FDA approved as the standard of care for advanced HCC. Due to recent emergence of resistance to sorafenib, second-line therapies with other targets are highly desirable.

 The chaperone-kinome pathway is attractive as a therapeutic target in cancer. In this pathway, the heat shock protein 90 (HSP90) cooperates with its molecular co-chaperone
25 CDC37 to regulate the folding, maturation, stabilization, and phosphorylation of a wide array of protein kinases, which are important mediators of signal transduction and cell growth in human cancers. HSP90 has been recognized as a key facilitator of oncogene addiction and a promising therapeutic target in cancers, with several HSP90 inhibitors in preclinical and clinical evaluation for cancer therapy. Current HSP90 inhibitors interact with the N-terminal ATP-binding pocket
30 and block ATP binding to stop the chaperone cycle, thereby leading to client protein degradation.

 On the contrary, disrupting the HSP90 and CDC37 chaperone complex has several advantages over targeting HSP90. For example, it obviates the undesirable induction of the anti-apoptotic heat shock response seen with HSP90 inhibition. CDC37 is the key permissive

factor in cell transformation caused by oncogenic protein kinases. The central segment of CDC37 associates with the N-terminal ATPase domain of HSP90, and the N-terminal segment of CDC37 associates with its client protein kinases (e.g., Cdk4, EGFR, AKT, MEK1/2 and Raf family proteins).

5 The present disclosure provides compounds and methods of use in the treatment of cancer (e.g, hepatocellular carcinoma).

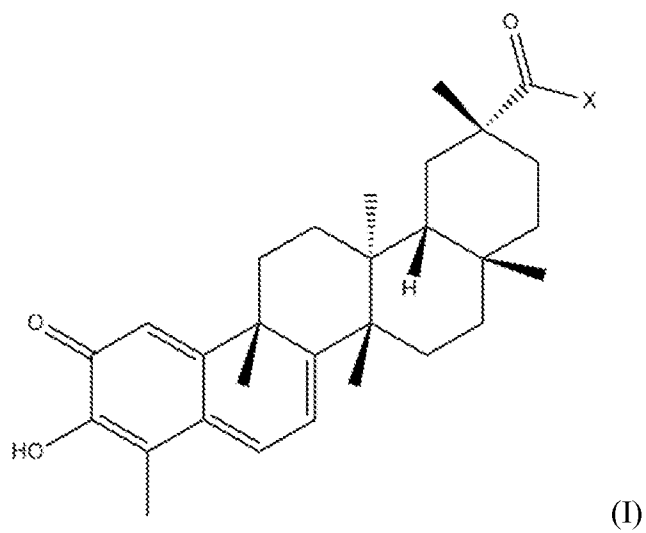
Publications

10 Abbas et al., Clin Cancer Res. 2007 Nov 15;13(22 Pt 1):6769-78; Casas et. al., Cancer Genet Cytogenet. 2003 146(2):89-101; Eum et al, Anticancer Drugs. 2011 Sep;22(8):763-73; Gray et. al., Nat Rev Cancer. 2008 8(7):491-495; Gray et. al., Cancer Res. 2007 67(24):11942-11950; Katayama et. al., Int J Oncol. 2004 25(3):579-595; Kim et. al., Curr Top Med Chem. 2009 9(15):1479-1492; Llovet et al., N Engl J Med. 2008 359(4):378-390; Marino-Enriquez et. al., Oncogene. 2014 Apr 3;33(14):1872-6; Mu et al, Asian Pac J Cancer Prev. 2012;13(4):1097-104; Neckers et. al., Clin Cancer Res. 2012 18(1):64-76; Pascale et. al., Hepatology. 2005 42(6):1310-1319; Sreeramulu et. al., Angew Chem Int Ed Engl. 2009 48(32):5853-5855; Stepanova et. al., Oncogene. 2000 19(18):2186-2193; Thompson et. al., Hum Pathol. 2005 36(5):494-504; Trepel et. al., Nat Rev Cancer. 2010 10(8):537-549; Villanueva and Llovet, Gastroenterology. 2011; 140(5):1410-1426; Wang et al., PLoS One. 2012;7(8):e43826. Epub 2012 Aug 28; Zaarur et. al., Cancer Res. 2006 66(3):1783-1791. U.S. patent numbers: 7,888,355 and 7,776,894. U.S. patent application number: 2011166216.

SUMMARY

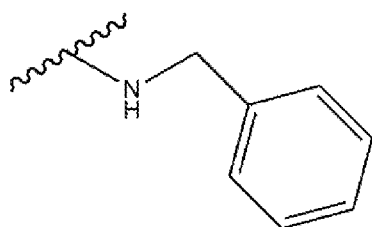
25 This disclosure concerns compounds which are useful as HSP90/CDC37 antagonists and are thus useful for treating a variety of diseases and disorders that are mediated or sustained through the interaction of HSP90 and CDC37. This disclosure also relates to pharmaceutical compositions that include these compounds, including formulations providing an effective dose, e.g. a unit dose, of the compound for treatment of cancer, including 30 without limitation carcinomas; methods of using these compounds in the treatment of various diseases and disorders, and processes for preparing these compounds.

Embodiments of the chemical structures are provided throughout the disclosure. By way of example, such compounds are represented by the following formula (I):



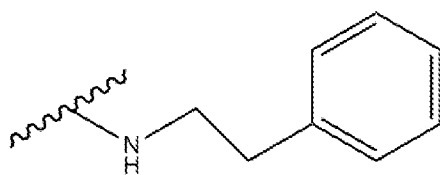
wherein

X is selected from:

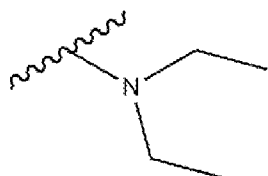


5

(compound 1; cel-D1);

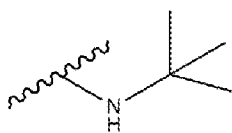


(compound 2; cel-D2);

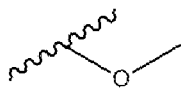


(compound 3; cel-D3);

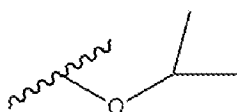
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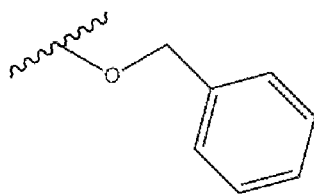
(compound 4; cel-D4);



(compound 5; cel-5);



5 (compound 6; cel-D6); and



(compound 7; cel-D7).

10 For example, aspects of the present disclosure include a compound, and formulations thereof, selected from:

Compound 1 (cel-D1): (2R,4aS,6aR,6aS,14aS,14bR)-N-benzyl-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxamide;

15 Compound 2 (cel-D2): (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-N-phenethyl-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 3 (cel-D3): (2R,4aS,6aS,12bR,14aS,14bR)-N,N-diethyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

20 Compound 4 (cel-D4): (2R,4aS,6aS,12bR,14aS,14bR)-N-tert-butyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 5 (cel-D5): methyl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-

2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahdropicene-2-carboxylate;

Compound 6 (cel-D6): propan-2-yl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahdropicene-2-carboxylate; and

Compound 7 (cel-D7): benzyl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-
5 2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahdropicene-2-carboxylate.

Aspects of the present disclosure include a pharmaceutical composition comprising a compound as described above and a pharmaceutically acceptable carrier, e.g. in a unit dose effective for treating cancer.

Aspects of the present disclosure include a method of antagonizing the interaction of
10 HSP90 with CDC37 in a biological sample or a patient, which method can include contacting the biological sample or administering to the patient a compound as described above. In some instances, antagonizing the interaction of HSP90 with CDC37 results in treatment of a disease or disorder that is mediated or sustained through the interaction of HSP90 with CDC37. In some instances, the disease or disorder is cancer (e.g., a carcinoma such as hepatocellular
15 carcinoma).

Aspects of the present disclosure include a method of antagonizing the interaction of HSP90 with CDC37 in an individual having cancer, which method can include administering to the individual, at a dose effective for antagonizing the interaction of HSP90 with CDC37 in cancer cells, a compound as described above. In some instances, the cancer is a carcinoma
20 (e.g., hepatocellular carcinoma).

Aspects of the present disclosure include a method of treating an individual having cancer, which method can include administering to the individual, at a dose effective for reducing the number of cancer cells in the individual, a compound as described above. In some instances, the cancer is a carcinoma (e.g., hepatocellular carcinoma). Such a method may
25 comprise (i) identifying a patient having hepatocellular cancer; and administering to the individual an effective dose of a compound described above, optionally in combination with additional chemotherapeutic agent(s).

Another aspect of the present invention relates to the use of a compound described above in the manufacture of a medicament for the treatment of cancer, including hepatocellular
30 carcinoma, wherein the medicament is administered to a patient in an effective dose, optionally in combination with additional chemotherapeutic agent(s).

Still another aspect of the present invention provides a kit for treatment of cancer. The kit includes a compound described herein, in an amount sufficient to reduce the growth of the cancer cells.

Aspects of the present disclosure further include a method for making Compound 2, Compound 3, or Compound 4, where the method includes contacting (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylic acid with phenethylamine (2-phenylethylamine), diethylamine (ethanamine), or tert-butylamine (2-propanamine). In some cases, the reaction is run in a polar aprotic solvent. In some cases, the reaction is run in the presence of a coupling reagent and a base.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. It is emphasized that, according to common practice, the various features of the drawings are not to scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

Figure 1. Celastrol and its derivatives disrupt HSP90/CDC37 interaction in HCC cells. HepG2 cells were incubated with 5 μ M of each compound and the same volume of DMSO as negative control for 6 hours. HSP90/CDC37 complex was then pulled down by anti-CDC37 antibody in the cell lysates. Anti-HSP90 antibody was used to detect the HSP90 protein in the complex. The lysates were used to detect HSP90, CDC37, and GAPDH (loading control).

Figures 2A-C. Celastrol and its derivatives are preferentially inhibited viability of HCC cells compared to normal hepatocytes. **(A).** CDC37 and GAPDH (loading control) expression were determined by Western Blot using specific antibodies in HCC cells (HepG2, Huh7, and Hep3B) and normal hepatocytes (Hu8114, Hu4175, and Hu8130). **(B).** Phase-contrast microscopic examination of the effect of cel-D7 (5 μ M) on HepG2 cells and normal hepatocytes Hu4175. **(C).** Celastrol and its derivatives (5 μ M each) induced apoptosis in Huh7 cells after 6 hours treatment. Cells were stained with TUNEL and DAPI as described under Materials and Methods to detect for apoptotic cells. Fluorescence labeling was visualized and photographed at 100x magnification.

Figures 3A-C. Celastrol and its derivatives induced degradation and inhibited phosphorylation of HSP90/CDC37 client proteins in HCC cell lines. HepG2, Huh7, and Hep3B cells were incubated for 6 hours with each compound (at 1 or 10 μ M) and CDC37,

HSP90/CDC37 client proteins and GAPDH (loading control) levels were determined by Western blotting using specific antibodies.

Figures 4A-F. Celastrol and its derivatives inhibited growth of orthotopic HCC patient-derived xenografts. **(A).** CDC37 and GAPDH (loading control) protein expressions in the tumor (T) and matched non-tumor liver (N) tissues of three HCC patients were determined by Western Blot. **(B, C, D).** Growth curves based on bioluminescence signals for HCC-1, HCC-2, and HCC-3 during the 3-week treatment period for each compound and saline control. *P < 0.05 for all compounds vs. saline control group (n=5 each). **(E).** Tumor volumes of HCC-1, HCC-2, and HCC-3 xenografts during the 3-week treatment period for each compound and saline control. *P < 0.05 for all compounds vs. saline control group (n=5 each). **(F).** Representative body weight curve of mice bearing HCC-3 xenografts during the 3-week treatment period with all four compounds.

Figure 5. Celastrol and its derivatives induced apoptosis (TUNEL Assay) in orthotopic HCC patient-derived xenografts. Representative images are shown for HCC-3 xenografts after 3 weeks treatment of each compound and saline control (200x magnification). Apoptotic cells are defined by cells with brown nucleic staining..

Figure 6. Celastrol and its derivatives induced degradation and inhibited phosphorylation of HSP90/CDC37 client proteins in orthotopic HCC patient-derived xenografts. Representative Western Blot results are shown for client proteins and their phosphorylation levels in two random tumors (T1 and T2) of HCC-3 xenografts after 3 weeks of treatment with each compound and saline control. GAPDH was used as a loading control.

Figure 7 presents a table of structures and activities of celastrol and subject compounds.

Figures 8A-D. Celastrol and its derivatives preferentially inhibited viability of HCC cells compared to normal hepatocytes. Cell viability assays based on ATP release were used to determine the cytotoxicity of celastrol and its derivatives on three human HCC cell lines cells (HepG2, Huh7, and Hep3B) and normal hepatocytes (Hu8114, Hu4175, and Hu8130) following 72 hours of treatment. Results are presented as mean $\pm$ SD (error bars). Relative ATP activity is proportional to the number of viable cells. The values of luciferase activity were normalized and compared with the DMSO control value, which was set at 100% cell viability. Three independent experiments were done, each in triplicates.

Figures 9A-F depict bioluminescence images of three HCC patient-derived xenograft models (mice) shown before treatment and after 3 weeks of treatment with celastrol or its derivatives. Orthotopic liver tumor models derived from human HCC patient specimens (HCC-1,

HCC-2, HCC-3) expressing a trifusion reporter gene were given intravenous injection of celastrol or its derivatives, and the tumor growth was monitored weekly using the Xenogen IVIS 100 imaging system.

Figures 10A-B depict synthetic schemes for (A) cel-D2 and (B) cel-D7. DIPEA:

5 Diisopropylethylamine; PyBOP: (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; DMF: Dimethylformamide; NaHCO₃:Sodium bicarbonate.

Figure 11 depicts ¹H NMR data for cel-D2

Figure 12 depicts ¹³C NMR data for cel-D7

Figure 13 depicts ¹³C NMR data for cel-D2

10 **Figure 14** depicts ¹H NMR data for cel-D7

Figure 15 depicts high resolution mass spectrometry data for cel-D2

Figure 16 depicts high resolution mass spectrometry data for cel-D7

DETAILED DESCRIPTION

15 This disclosure concerns compounds which are useful as HSP90/CDC37 antagonists and are thus useful for treating a variety of diseases and disorders that are mediated or sustained through the interaction of HSP90 and CDC37. This disclosure also relates to pharmaceutical compositions that include these compounds, methods of using these compounds in the treatment of various diseases and disorders, and processes for preparing
20 these compounds.

Before the present methods and compositions are described, it is to be understood that this invention is not limited to particular method or composition described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of
25 describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between
30 any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range

includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. It is understood that the present disclosure supercedes any disclosure of an incorporated publication to the extent there is a contradiction.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the peptide" includes reference to one or more peptides and equivalents thereof, e.g. polypeptides, known to those skilled in the art, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed

Except as otherwise noted, the methods and techniques of the present embodiments are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, e.g., Loudon, Organic Chemistry, Fourth Edition, New York: Oxford University Press, 2002, pp. 360-361, 1084-1085; Smith and March, March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, Fifth Edition, Wiley-Interscience, 2001; or Vogel, A Textbook of Practical Organic Chemistry, Including Qualitative Organic Analysis, Fourth Edition, New York: Longman, 1978.

The nomenclature used herein to name the subject compounds is illustrated in the Examples herein. This nomenclature has generally been derived using the commercially-available AutoNom software (MDL, San Leandro, CA.).

Definitions

5 The following terms have the following meanings unless otherwise indicated. Any undefined terms have their art recognized meanings.

“Alkyl” refers to monovalent saturated aliphatic hydrocarbyl groups having from 1 to 10 carbon atoms and preferably 1 to 6 carbon atoms. This term includes, by way of example, linear and branched hydrocarbyl groups such as methyl (CH_3 -), ethyl (CH_3CH_2 -), n-propyl ($\text{CH}_3\text{CH}_2\text{CH}_2$ -), isopropyl ($(\text{CH}_3)_2\text{CH}$ -), n-butyl ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2$ -), isobutyl ($(\text{CH}_3)_2\text{CHCH}_2$ -), sec-butyl ($(\text{CH}_3)(\text{CH}_3\text{CH}_2)\text{CH}$ -), t-butyl ($(\text{CH}_3)_3\text{C}$ -), n-pentyl ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ -), and neopentyl ($(\text{CH}_3)_3\text{CCH}_2$ -).

The term “substituted alkyl” refers to an alkyl group as defined herein wherein one or more carbon atoms in the alkyl chain have been optionally replaced with a heteroatom such as -O-, -N-, -S-, -S(O)_n- (where n is 0 to 2), -NR- (where R is hydrogen or alkyl) and having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-aryl, -SO₂-heteroaryl, and -NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

25 “Alkylene” refers to divalent aliphatic hydrocarbyl groups preferably having from 1 to 6 and more preferably 1 to 3 carbon atoms that are either straight-chained or branched, and which are optionally interrupted with one or more groups selected from -O-, -NR¹⁰-, -NR¹⁰C(O)-, -C(O)NR¹⁰- and the like. This term includes, by way of example, methylene (-CH₂-), ethylene (-CH₂CH₂-), n-propylene (-CH₂CH₂CH₂-), iso-propylene (-CH₂CH(CH₃)-), (-C(CH₃)₂CH₂CH₂-), (-C(CH₃)₂CH₂C(O)-), (-C(CH₃)₂CH₂C(O)NH-), (-CH(CH₃)CH₂-), and the like.

30 “Substituted alkylene” refers to an alkylene group having from 1 to 3 hydrogens replaced with substituents as described for carbons in the definition of “substituted” below.

The term “alkane” refers to alkyl group and alkylene group, as defined herein.

The term "alkylaminoalkyl", "alkylaminoalkenyl" and "alkylaminoalkynyl" refers to the groups R'NHR"- where R' is alkyl group as defined herein and R" is alkylene, alkenylene or alkynylene group as defined herein.

5 The term "alkaryl" or "aralkyl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl where alkylene, substituted alkylene and aryl are defined herein.

"Alkoxy" refers to the group -O-alkyl, wherein alkyl is as defined herein. Alkoxy includes, by way of example, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, t-butoxy, sec-butoxy, n-pentoxy, and the like. The term "alkoxy" also refers to the groups alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkenyl, cycloalkyl, cycloalkenyl, and
10 alkynyl are as defined herein.

The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

15 The term "alkoxyamino" refers to the group -NH-alkoxy, wherein alkoxy is defined herein.

The term "haloalkoxy" refers to the groups alkyl-O- wherein one or more hydrogen atoms on the alkyl group have been substituted with a halo group and include, by way of examples, groups such as trifluoromethoxy, and the like.

20 The term "haloalkyl" refers to a substituted alkyl group as described above, wherein one or more hydrogen atoms on the alkyl group have been substituted with a halo group. Examples of such groups include, without limitation, fluoroalkyl groups, such as trifluoromethyl, difluoromethyl, trifluoroethyl and the like.

The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl, and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein.

The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein.

30 "Alkenyl" refers to straight chain or branched hydrocarbonyl groups having from 2 to 6 carbon atoms and preferably 2 to 4 carbon atoms and having at least 1 and preferably from 1 to 2 sites of double bond unsaturation. This term includes, by way of example, bi-vinyl, allyl, and but-3-en-1-yl. Included within this term are the cis and trans isomers or mixtures of these isomers.

The term “substituted alkenyl” refers to an alkenyl group as defined herein having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

“Alkynyl” refers to straight or branched monovalent hydrocarbyl groups having from 2 to 6 carbon atoms and preferably 2 to 3 carbon atoms and having at least 1 and preferably from 1 to 2 sites of triple bond unsaturation. Examples of such alkynyl groups include acetylenyl (-C≡CH), and propargyl (-CH₂C≡CH).

The term “substituted alkynyl” refers to an alkynyl group as defined herein having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl, and -SO₂-heteroaryl.

“Alkynyloxy” refers to the group -O-alkynyl, wherein alkynyl is as defined herein. Alkynyloxy includes, by way of example, ethynyloxy, propynyloxy, and the like.

“Acyl” refers to the groups H-C(O)-, alkyl-C(O)-, substituted alkyl-C(O)-, alkenyl-C(O)-, substituted alkenyl-C(O)-, alkynyl-C(O)-, substituted alkynyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, substituted aryl-C(O)-, heteroaryl-C(O)-, substituted heteroaryl-C(O)-, heterocyclyl-C(O)-, and substituted heterocyclyl-C(O)-, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein. For example, acyl includes the “acetyl” group CH₃C(O)-

“Acylamino” refers to the groups -NR²⁰C(O)alkyl, -NR²⁰C(O)substituted alkyl, N R²⁰C(O)cycloalkyl, -NR²⁰C(O)substituted cycloalkyl, -

NR²⁰C(O)cycloalkenyl, -NR²⁰C(O)substituted cycloalkenyl, -NR²⁰C(O)alkenyl, -
 NR²⁰C(O)substituted alkenyl, -NR²⁰C(O)alkynyl, -NR²⁰C(O)substituted
 alkynyl, -NR²⁰C(O)aryl, -NR²⁰C(O)substituted aryl, -NR²⁰C(O)heteroaryl, -NR²⁰C(O)substituted
 heteroaryl, -NR²⁰C(O)heterocyclic, and -NR²⁰C(O)substituted heterocyclic, wherein R²⁰ is
 5 hydrogen or alkyl and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl,
 substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl,
 substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are
 as defined herein.

“Aminocarbonyl” or the term “aminoacyl” refers to the group -C(O)NR²¹R²², wherein R²¹
 10 and R²² independently are selected from the group consisting of hydrogen, alkyl, substituted
 alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl,
 substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl,
 heterocyclic, and substituted heterocyclic and where R²¹ and R²² are optionally joined together
 with the nitrogen bound thereto to form a heterocyclic or substituted heterocyclic group, and
 15 wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl,
 cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl,
 heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined
 herein.

“Aminocarbonylamino” refers to the group -NR²¹C(O)NR²²R²³ where R²¹, R²², and R²³
 20 are independently selected from hydrogen, alkyl, aryl or cycloalkyl, or where two R groups are
 joined to form a heterocyclyl group.

The term “alkoxycarbonylamino” refers to the group -NRC(O)OR where each R is
 independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclyl wherein alkyl,
 substituted alkyl, aryl, heteroaryl, and heterocyclyl are as defined herein.

25 The term “acyloxy” refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-,
 cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and
 heterocyclyl-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl,
 heteroaryl, and heterocyclyl are as defined herein.

“Aminosulfonyl” refers to the group -SO₂NR²¹R²², wherein R²¹ and R²² independently are
 30 selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted
 alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl,
 cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl, heterocyclic,
 substituted heterocyclic and where R²¹ and R²² are optionally joined together with the nitrogen
 bound thereto to form a heterocyclic or substituted heterocyclic group and alkyl, substituted

alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

“Sulfonylamino” refers to the group $\text{-NR}^{21}\text{SO}_2\text{R}^{22}$, wherein R^{21} and R^{22} independently are
 5 selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic and where R^{21} and R^{22} are optionally joined together with the atoms bound thereto to form a heterocyclic or substituted heterocyclic group, and wherein alkyl,
 10 substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

“Aryl” or “Ar” refers to a monovalent aromatic carbocyclic group of from 6 to 18 carbon atoms having a single ring (such as is present in a phenyl group) or a ring system having
 15 multiple condensed rings (examples of such aromatic ring systems include naphthyl, anthryl and indanyl) which condensed rings may or may not be aromatic, provided that the point of attachment is through an atom of an aromatic ring. This term includes, by way of example, phenyl and naphthyl. Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents, or from 1 to 3
 20 substituents, selected from acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halogen, nitro, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, aminoacyloxy, oxyacylamino, thioalkoxy,
 25 substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl , $\text{-SO-substituted alkyl}$, -SO-aryl , -SO-heteroaryl , $\text{-SO}_2\text{-alkyl}$, $\text{-SO}_2\text{-substituted alkyl}$, $\text{-SO}_2\text{-aryl}$, $\text{-SO}_2\text{-heteroaryl}$ and trihalomethyl.

“Aryloxy” refers to the group -O-aryl , wherein aryl is as defined herein, including, by way of example, phenoxy, naphthoxy, and the like, including optionally substituted aryl groups as also defined herein.

30 “Amino” refers to the group -NH_2 .

The term “substituted amino” refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl,

substituted alkynyl, aryl, heteroaryl, and heterocyclyl provided that at least one R is not hydrogen.

The term "azido" refers to the group $-N_3$.

"Carboxyl," "carboxy" or "carboxylate" refers to $-CO_2H$ or salts thereof.

5 "Carboxyl ester" or "carboxy ester" or the terms "carboxyalkyl" or "carboxylalkyl" refers to the groups $-C(O)O$ -alkyl, $-C(O)O$ -substituted alkyl, $-C(O)O$ -alkenyl, $-C(O)O$ -substituted alkenyl, $-C(O)O$ -alkynyl, $-C(O)O$ -substituted alkynyl, $-C(O)O$ -aryl, $-C(O)O$ -substituted aryl, $-C(O)O$ -cycloalkyl, $-C(O)O$ -substituted cycloalkyl, $-C(O)O$ -cycloalkenyl, $-C(O)O$ -substituted cycloalkenyl, $-C(O)O$ -heteroaryl, $-C(O)O$ -substituted heteroaryl, $-C(O)O$ -heterocyclic, and $-C(O)O$ -substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

15 "(Carboxyl ester)oxy" or "carbonate" refers to the groups $-O-C(O)O$ -alkyl, $-O-C(O)O$ -substituted alkyl, $-O-C(O)O$ -alkenyl, $-O-C(O)O$ -substituted alkenyl, $-O-C(O)O$ -alkynyl, $-O-C(O)O$ -substituted alkynyl, $-O-C(O)O$ -aryl, $-O-C(O)O$ -substituted aryl, $-O-C(O)O$ -cycloalkyl, $-O-C(O)O$ -substituted cycloalkyl, $-O-C(O)O$ -cycloalkenyl, $-O-C(O)O$ -substituted cycloalkenyl, $-O-C(O)O$ -heteroaryl, $-O-C(O)O$ -substituted heteroaryl, $-O-C(O)O$ -heterocyclic, and $-O-C(O)O$ -substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

"Cyano" or "nitrile" refers to the group $-CN$.

25 "Cycloalkyl" refers to cyclic alkyl groups of from 3 to 10 carbon atoms having single or multiple cyclic rings including fused, bridged, and spiro ring systems. Examples of suitable cycloalkyl groups include, for instance, adamantyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl and the like. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

30 The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkyl, substituted alkyl, alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy,

thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

5 “Cycloalkenyl” refers to non-aromatic cyclic alkyl groups of from 3 to 10 carbon atoms having single or multiple rings and having at least one double bond and preferably from 1 to 2 double bonds.

 The term “substituted cycloalkenyl” refers to cycloalkenyl groups having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

 “Cycloalkynyl” refers to non-aromatic cycloalkyl groups of from 5 to 10 carbon atoms having single or multiple rings and having at least one triple bond.

 “Cycloalkoxy” refers to -O-cycloalkyl.

 “Cycloalkenyloxy” refers to -O-cycloalkenyl.

20 “Halo” or “halogen” refers to fluoro, chloro, bromo, and iodo.

 “Hydroxy” or “hydroxyl” refers to the group -OH.

 “Heteroaryl” refers to an aromatic group of from 1 to 15 carbon atoms, such as from 1 to 10 carbon atoms and 1 to 10 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur within the ring. Such heteroaryl groups can have a single ring (such as, pyridinyl, imidazolyl or furyl) or multiple condensed rings in a ring system (for example as in groups such as, indolizinyl, quinolinyl, benzofuran, benzimidazolyl or benzothienyl), wherein at least one ring within the ring system is aromatic and at least one ring within the ring system is aromatic, provided that the point of attachment is through an atom of an aromatic ring. In certain embodiments, the nitrogen and/or sulfur ring atom(s) of the heteroaryl group are optionally oxidized to provide for the N-oxide (N→O), sulfinyl, or sulfonyl moieties. This term includes, by way of example, pyridinyl, pyrrolyl, indolyl, thiophenyl, and furanyl. Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents, or from 1 to 3 substituents, selected from acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted

alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halogen, nitro, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl, and trihalomethyl.

The term "heteroaralkyl" refers to the groups -alkylene-heteroaryl where alkylene and heteroaryl are defined herein. This term includes, by way of example, pyridylmethyl, pyridylethyl, indolylmethyl, and the like.

"Heteroaryloxy" refers to -O-heteroaryl.

"Heterocycle," "heterocyclic," "heterocycloalkyl," and "heterocyclyl" refer to a saturated or unsaturated group having a single ring or multiple condensed rings, including fused bridged and spiro ring systems, and having from 3 to 20 ring atoms, including 1 to 10 hetero atoms. These ring atoms are selected from the group consisting of nitrogen, sulfur, or oxygen, wherein, in fused ring systems, one or more of the rings can be cycloalkyl, aryl, or heteroaryl, provided that the point of attachment is through the non-aromatic ring. In certain embodiments, the nitrogen and/or sulfur atom(s) of the heterocyclic group are optionally oxidized to provide for the N-oxide, -S(O)-, or -SO₂- moieties.

Examples of heterocycles and heteroaryls include, but are not limited to, azetidine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, dihydroindole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quiazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, phthalimide, 1,2,3,4-tetrahydroisoquinoline, 4,5,6,7-tetrahydrobenzo[b]thiophene, thiazole, thiazolidine, thiophene, benzo[b]thiophene, morpholinyl, thiomorpholinyl (also referred to as thiamorpholinyl), 1,1-dioxothiomorpholinyl, piperidinyl, pyrrolidine, tetrahydrofuranyl, and the like.

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino,

alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl, -SO₂-heteroaryl, and fused heterocycle.

"Heterocycloxy" refers to the group -O-heterocyclyl.

The term "heterocyclylthio" refers to the group heterocyclic-S-.

5 The term "heterocyclene" refers to the diradical group formed from a heterocycle, as defined herein.

The term "hydroxyamino" refers to the group -NHOH.

"Nitro" refers to the group -NO₂.

"Oxo" refers to the atom (=O).

10 "Sulfonyl" refers to the group SO₂-alkyl, SO₂-substituted alkyl, SO₂-alkenyl, SO₂-substituted alkenyl, SO₂-cycloalkyl, SO₂-substituted cycloalkyl, SO₂-cycloalkenyl, SO₂-substituted cycloalkenyl, SO₂-aryl, SO₂-substituted aryl, SO₂-heteroaryl, SO₂-substituted heteroaryl, SO₂-heterocyclic, and SO₂-substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, 15 cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein. Sulfonyl includes, by way of example, methyl-SO₂-, phenyl-SO₂-, and 4-methylphenyl-SO₂-.

"Sulfonyloxy" refers to the group -OSO₂-alkyl, OSO₂-substituted alkyl, OSO₂-alkenyl, OSO₂-substituted alkenyl, OSO₂-cycloalkyl, OSO₂-substituted cycloalkyl, OSO₂-cycloalkenyl, 20 OSO₂-substituted cycloalkenyl, OSO₂-aryl, OSO₂-substituted aryl, OSO₂-heteroaryl, OSO₂-substituted heteroaryl, OSO₂-heterocyclic, and OSO₂ substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

25 The term "aminocarbonyloxy" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Thiol" refers to the group -SH.

"Thioxo" or the term "thioketo" refers to the atom (=S).

30 "Alkylthio" or the term "thioalkoxy" refers to the group -S-alkyl, wherein alkyl is as defined herein. In certain embodiments, sulfur may be oxidized to -S(O)-. The sulfoxide may exist as one or more stereoisomers.

The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

The term “thioaryloxy” refers to the group aryl-S- wherein the aryl group is as defined herein including optionally substituted aryl groups also defined herein.

The term “thioheteroaryloxy” refers to the group heteroaryl-S- wherein the heteroaryl group is as defined herein including optionally substituted aryl groups as also defined herein.

5 The term “thioheterocycloxy” refers to the group heterocyclyl-S- wherein the heterocyclyl group is as defined herein including optionally substituted heterocyclyl groups as also defined herein.

In addition to the disclosure herein, the term “substituted,” when used to modify a specified group or radical, can also mean that one or more hydrogen atoms of the specified group or radical are each, independently of one another, replaced with the same or different
10 substituent groups as defined below.

In addition to the groups disclosed with respect to the individual terms herein, substituent groups for substituting for one or more hydrogens (any two hydrogens on a single carbon can be replaced with =O, =NR⁷⁰, =N-OR⁷⁰, =N₂ or =S) on saturated carbon atoms in the specified
15 group or radical are, unless otherwise specified, -R⁶⁰, halo, =O, -OR⁷⁰, -SR⁷⁰, -NR⁸⁰R⁸⁰, trihalomethyl, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -SO₂R⁷⁰, -SO₂O⁻M⁺, -SO₂OR⁷⁰, -OSO₂R⁷⁰, -OSO₂O⁻M⁺, -OSO₂OR⁷⁰, -P(O)(O⁻)₂(M⁺)₂, -P(O)(OR⁷⁰)O⁻M⁺, -P(O)(OR⁷⁰)₂, -C(O)R⁷⁰, -C(S)R⁷⁰, -C(NR⁷⁰)R⁷⁰, -C(O)O⁻M⁺, -C(O)OR⁷⁰, -C(S)OR⁷⁰, -C(O)NR⁸⁰R⁸⁰, -C(NR⁷⁰)NR⁸⁰R⁸⁰, -OC(O)R⁷⁰, -OC(S)R⁷⁰, -OC(O)O⁻M⁺, -OC(O)OR⁷⁰, -OC(S)OR⁷⁰, -NR⁷⁰C(O)R⁷⁰, -NR⁷⁰C(S)R⁷⁰, -NR⁷⁰CO₂⁻M⁺, -NR⁷⁰CO₂R⁷⁰, -NR⁷⁰C(S)OR⁷⁰, -NR⁷⁰C(O)NR⁸⁰R⁸⁰, -NR⁷⁰C(NR⁷⁰)R⁷⁰
20 and -NR⁷⁰C(NR⁷⁰)NR⁸⁰R⁸⁰, where R⁶⁰ is selected from the group consisting of optionally substituted alkyl, cycloalkyl, heteroalkyl, heterocycloalkylalkyl, cycloalkylalkyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl, each R⁷⁰ is independently hydrogen or R⁶⁰; each R⁸⁰ is
25 independently R⁷⁰ or alternatively, two R⁸⁰'s, taken together with the nitrogen atom to which they are bonded, form a 5-, 6- or 7-membered heterocycloalkyl which may optionally include from 1 to 4 of the same or different additional heteroatoms selected from the group consisting of O, N and S, of which N may have -H or C₁-C₃ alkyl substitution; and each M⁺ is a counter ion with a net single positive charge. Each M⁺ may independently be, for example, an alkali ion, such as
30 K⁺, Na⁺, Li⁺; an ammonium ion, such as ⁺N(R⁶⁰)₄; or an alkaline earth ion, such as [Ca²⁺]_{0.5}, [Mg²⁺]_{0.5}, or [Ba²⁺]_{0.5} (“subscript 0.5 means that one of the counter ions for such divalent alkali earth ions can be an ionized form of a compound of the invention and the other a typical counter ion such as chloride, or two ionized compounds disclosed herein can serve as counter ions for such divalent alkali earth ions, or a doubly ionized compound of the invention can serve as the

counter ion for such divalent alkali earth ions). As specific examples, $-\text{NR}^{80}\text{R}^{80}$ is meant to include $-\text{NH}_2$, $-\text{NH}$ -alkyl, *N*-pyrrolidinyl, *N*-piperazinyl, 4*N*-methyl-piperazin-1-yl and *N*-morpholinyl.

In addition to the disclosure herein, substituent groups for hydrogens on unsaturated carbon atoms in “substituted” alkene, alkyne, aryl and heteroaryl groups are, unless otherwise specified, $-\text{R}^{60}$, halo, $-\text{O}^-\text{M}^+$, $-\text{OR}^{70}$, $-\text{SR}^{70}$, $-\text{S}^-\text{M}^+$, $-\text{NR}^{80}\text{R}^{80}$, trihalomethyl, $-\text{CF}_3$, $-\text{CN}$, $-\text{OCN}$, $-\text{SCN}$, $-\text{NO}$, $-\text{NO}_2$, $-\text{N}_3$, $-\text{SO}_2\text{R}^{70}$, $-\text{SO}_3^-$ M^+ , $-\text{SO}_3\text{R}^{70}$, $-\text{OSO}_2\text{R}^{70}$, $-\text{OSO}_3^-\text{M}^+$, $-\text{OSO}_3\text{R}^{70}$, $-\text{PO}_3^{2-}(\text{M}^+)_2$, $-\text{P}(\text{O})(\text{OR}^{70})\text{O}^-$ M^+ , $-\text{P}(\text{O})(\text{OR}^{70})_2$, $-\text{C}(\text{O})\text{R}^{70}$, $-\text{C}(\text{S})\text{R}^{70}$, $-\text{C}(\text{NR}^{70})\text{R}^{70}$, $-\text{CO}_2^-$ M^+ , $-\text{CO}_2\text{R}^{70}$, $-\text{C}(\text{S})\text{OR}^{70}$, $-\text{C}(\text{O})\text{NR}^{80}\text{R}^{80}$, $-\text{C}(\text{NR}^{70})\text{NR}^{80}\text{R}^{80}$, $-\text{OC}(\text{O})\text{R}^{70}$, $-\text{OC}(\text{S})\text{R}^{70}$, $-\text{OCO}_2^-$ M^+ , $-\text{OCO}_2\text{R}^{70}$, $-\text{OC}(\text{S})\text{OR}^{70}$, $-\text{NR}^{70}\text{C}(\text{O})\text{R}^{70}$, $-\text{NR}^{70}\text{C}(\text{S})\text{R}^{70}$, $-\text{NR}^{70}\text{CO}_2^-$ M^+ , $-\text{NR}^{70}\text{CO}_2\text{R}^{70}$, $-\text{NR}^{70}\text{C}(\text{S})\text{OR}^{70}$, $-\text{NR}^{70}\text{C}(\text{O})\text{NR}^{80}\text{R}^{80}$, $-\text{NR}^{70}\text{C}(\text{NR}^{70})\text{R}^{70}$ and $-\text{NR}^{70}\text{C}(\text{NR}^{70})\text{NR}^{80}\text{R}^{80}$, where R^{60} , R^{70} , R^{80} and M^+ are as previously defined, provided that in case of substituted alkene or alkyne, the substituents are not $-\text{O}^-\text{M}^+$, $-\text{OR}^{70}$, $-\text{SR}^{70}$, or $-\text{S}^-\text{M}^+$.

In addition to the groups disclosed with respect to the individual terms herein, substituent groups for hydrogens on nitrogen atoms in “substituted” heteroalkyl and cycloheteroalkyl groups are, unless otherwise specified, $-\text{R}^{60}$, $-\text{O}^-\text{M}^+$, $-\text{OR}^{70}$, $-\text{SR}^{70}$, $-\text{S}^-\text{M}^+$, $-\text{NR}^{80}\text{R}^{80}$, trihalomethyl, $-\text{CF}_3$, $-\text{CN}$, $-\text{NO}$, $-\text{NO}_2$, $-\text{S}(\text{O})_2\text{R}^{70}$, $-\text{S}(\text{O})_2\text{O}^-\text{M}^+$, $-\text{S}(\text{O})_2\text{OR}^{70}$, $-\text{OS}(\text{O})_2\text{R}^{70}$, $-\text{OS}(\text{O})_2\text{O}^-$ M^+ , $-\text{OS}(\text{O})_2\text{OR}^{70}$, $-\text{P}(\text{O})(\text{O}^-)_2(\text{M}^+)_2$, $-\text{P}(\text{O})(\text{OR}^{70})\text{O}^-\text{M}^+$, $-\text{P}(\text{O})(\text{OR}^{70})(\text{OR}^{70})$, $-\text{C}(\text{O})\text{R}^{70}$, $-\text{C}(\text{S})\text{R}^{70}$, $-\text{C}(\text{NR}^{70})\text{R}^{70}$, $-\text{C}(\text{O})\text{OR}^{70}$, $-\text{C}(\text{S})\text{OR}^{70}$, $-\text{C}(\text{O})\text{NR}^{80}\text{R}^{80}$, $-\text{C}(\text{NR}^{70})\text{NR}^{80}\text{R}^{80}$, $-\text{OC}(\text{O})\text{R}^{70}$, $-\text{OC}(\text{S})\text{R}^{70}$, $-\text{OC}(\text{O})\text{OR}^{70}$, $-\text{OC}(\text{S})\text{OR}^{70}$, $-\text{NR}^{70}\text{C}(\text{O})\text{R}^{70}$, $-\text{NR}^{70}\text{C}(\text{S})\text{R}^{70}$, $-\text{NR}^{70}\text{C}(\text{O})\text{OR}^{70}$, $-\text{NR}^{70}\text{C}(\text{S})\text{OR}^{70}$, $-\text{NR}^{70}\text{C}(\text{O})\text{NR}^{80}\text{R}^{80}$, $-\text{NR}^{70}\text{C}(\text{NR}^{70})\text{R}^{70}$ and $-\text{NR}^{70}\text{C}(\text{NR}^{70})\text{NR}^{80}\text{R}^{80}$, where R^{60} , R^{70} , R^{80} and M^+ are as previously defined.

In addition to the disclosure herein, in a certain embodiment, a group that is substituted has 1, 2, 3, or 4 substituents, 1, 2, or 3 substituents, 1 or 2 substituents, or 1 substituent.

It is understood that in all substituted groups defined above, polymers arrived at by defining substituents with further substituents to themselves (e.g., substituted aryl having a substituted aryl group as a substituent which is itself substituted with a substituted aryl group, which is further substituted by a substituted aryl group, etc.) are not intended for inclusion herein. In such cases, the maximum number of such substitutions is three. For example, serial substitutions of substituted aryl groups specifically contemplated herein are limited to substituted aryl-(substituted aryl)-substituted aryl.

Unless indicated otherwise, the nomenclature of substituents that are not explicitly defined herein are arrived at by naming the terminal portion of the functionality followed by the

adjacent functionality toward the point of attachment. For example, the substituent “arylalkyloxycarbonyl” refers to the group (aryl)-(alkyl)-O-C(O)-.

As to any of the groups disclosed herein which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the subject compounds include all stereochemical isomers arising from the substitution of these compounds.

The term “pharmaceutically acceptable salt” means a salt which is acceptable for administration to a patient, such as a mammal (salts with counterions having acceptable mammalian safety for a given dosage regime). Such salts can be derived from pharmaceutically acceptable inorganic or organic bases and from pharmaceutically acceptable inorganic or organic acids. “Pharmaceutically acceptable salt” refers to pharmaceutically acceptable salts of a compound, which salts are derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, formate, tartrate, besylate, mesylate, acetate, maleate, oxalate, and the like.

The term “salt thereof” means a compound formed when a proton of an acid is replaced by a cation, such as a metal cation or an organic cation and the like. Where applicable, the salt is a pharmaceutically acceptable salt, although this is not required for salts of intermediate compounds that are not intended for administration to a patient. By way of example, salts of the present compounds include those wherein the compound is protonated by an inorganic or organic acid to form a cation, with the conjugate base of the inorganic or organic acid as the anionic component of the salt.

“Solvate” refers to a complex formed by combination of solvent molecules with molecules or ions of the solute. The solvent can be an organic compound, an inorganic compound, or a mixture of both. Some examples of solvents include, but are not limited to, methanol, *N,N*-dimethylformamide, tetrahydrofuran, dimethylsulfoxide, and water. When the solvent is water, the solvate formed is a hydrate.

“Stereoisomer” and “stereoisomers” refer to compounds that have same atomic connectivity but different atomic arrangement in space. Stereoisomers include cis-trans isomers, *E* and *Z* isomers, enantiomers, and diastereomers.

“Tautomer” refers to alternate forms of a molecule that differ only in electronic bonding of atoms and/or in the position of a proton, such as enol-keto and imine-enamine tautomers, or the

tautomeric forms of heteroaryl groups containing a -N=C(H)-NH- ring atom arrangement, such as pyrazoles, imidazoles, benzimidazoles, triazoles, and tetrazoles. A person of ordinary skill in the art would recognize that other tautomeric ring atom arrangements are possible.

It will be appreciated that the term “or a salt or solvate or stereoisomer thereof” is intended to include all permutations of salts, solvates and stereoisomers, such as a solvate of a pharmaceutically acceptable salt of a stereoisomer of subject compound.

“Pharmaceutically effective amount” and “therapeutically effective amount” refer to an amount of a compound sufficient to treat a specified disorder or disease or one or more of its symptoms and/or to prevent the occurrence of the disease or disorder. In reference to tumorigenic proliferative disorders, a pharmaceutically or therapeutically effective amount comprises an amount sufficient to, among other things, cause the tumor to shrink or decrease the growth rate of the tumor.

The terms “recipient”, “individual”, “subject”, “host”, and “patient”, are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. “Mammal” for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, goats, pigs, etc. In some cases, the mammal is human.

In some cases, a target cell is an “inflicted” cell (e.g., a cell from an “inflicted” individual), where the term “inflicted” is used herein to refer to a subject with symptoms, an illness, or a disease that can be treated with a subject compound. An “inflicted” subject can have cancer (e.g., HCC) and/or can have other hyper-proliferative conditions, for example sclerosis, fibrosis, and the like, etc. “Inflicted cells” can be those cells that cause the symptoms, illness, or disease. As a non-limiting example, an inflicted cell of an inflicted patient can be a cancer cell (e.g., an hepatocellular carcinoma cell).

The terms “treatment”, “treating”, “treat” and the like are used herein to generally refer to obtaining a desired pharmacologic and/or physiologic effect. The effect can be prophylactic in terms of completely or partially preventing a disease or symptom(s) thereof and/or may be therapeutic in terms of a partial or complete stabilization or cure for a disease and/or adverse effect attributable to the disease. The term “treatment” encompasses any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease and/or symptom(s) from occurring in a subject who may be predisposed to the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease and/or symptom(s), i.e., arresting their development; or (c) relieving the disease symptom(s), i.e., causing regression of the disease

and/or symptom(s). Those in need of treatment include those already inflicted (e.g., those with cancer) as well as those in which prevention is desired (e.g., those with increased susceptibility to cancer).

A therapeutic treatment is one in which the subject is inflicted prior to administration and a prophylactic treatment is one in which the subject is not inflicted prior to administration. In some embodiments, the subject has an increased likelihood of becoming inflicted or is suspected of being inflicted prior to treatment. In some embodiments, the subject is suspected of having an increased likelihood of becoming inflicted.

The terms "co-administration", "co-administer", and "in combination with" include the administration of two or more therapeutic agents either simultaneously, concurrently or sequentially within no specific time limits. In one embodiment, the agents are present in the cell or in the subject's body at the same time or exert their biological or therapeutic effect at the same time. In one embodiment, the therapeutic agents are in the same composition or unit dosage form. In other embodiments, the therapeutic agents are in separate compositions or unit dosage forms. In certain embodiments, a first agent can be administered prior to (e.g., minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapeutic agent.

As used herein "cancer" includes any form of cancer, including but not limited to solid tumor cancers (e.g., lung, prostate, breast, bladder, colon, ovarian, pancreas, kidney, liver, glioblastoma, medulloblastoma, leiomyosarcoma, head & neck squamous cell carcinomas, melanomas, neuroendocrine; etc.) and liquid cancers (e.g., hematological cancers); carcinomas; soft tissue tumors; sarcomas; teratomas; melanomas; leukemias; lymphomas; and brain cancers, including minimal residual disease, and including both primary and metastatic tumors.

Carcinomas are malignancies that originate in the epithelial tissues. Epithelial cells cover the external surface of the body, line the internal cavities, and form the lining of glandular tissues. Examples of carcinomas include, but are not limited to: adenocarcinoma (cancer that begins in glandular (secretory) cells), e.g., cancers of the breast, pancreas, lung, prostate, and colon can be adenocarcinomas; adrenocortical carcinoma; hepatocellular carcinoma (HCC, malignant hepatoma); renal cell carcinoma; ovarian carcinoma; carcinoma in situ; ductal

carcinoma; carcinoma of the breast; basal cell carcinoma; squamous cell carcinoma; transitional cell carcinoma; colon carcinoma; nasopharyngeal carcinoma; multilocular cystic renal cell carcinoma; oat cell carcinoma; large cell lung carcinoma; small cell lung carcinoma; non-small cell lung carcinoma; and the like. Carcinomas may be found in prostate, pancreas, colon, brain
 5 (usually as secondary metastases), lung, breast, skin, etc.

Soft tissue tumors are a highly diverse group of rare tumors that are derived from connective tissue. Examples of soft tissue tumors include, but are not limited to: alveolar soft part sarcoma; angiomatoid fibrous histiocytoma; chondromyxoid fibroma; skeletal chondrosarcoma; extraskeletal myxoid chondrosarcoma; clear cell sarcoma; desmoplastic small
 10 round-cell tumor; dermatofibrosarcoma protuberans; endometrial stromal tumor; Ewing's sarcoma; fibromatosis (Desmoid); fibrosarcoma, infantile; gastrointestinal stromal tumor; bone giant cell tumor; tenosynovial giant cell tumor; inflammatory myofibroblastic tumor; uterine leiomyoma; leiomyosarcoma; lipoblastoma; typical lipoma; spindle cell or pleomorphic lipoma; atypical lipoma; chondroid lipoma; well-differentiated liposarcoma; myxoid/round cell
 15 liposarcoma; pleomorphic liposarcoma; myxoid malignant fibrous histiocytoma; high-grade malignant fibrous histiocytoma; myxofibrosarcoma; malignant peripheral nerve sheath tumor; mesothelioma; neuroblastoma; osteochondroma; osteosarcoma; primitive neuroectodermal tumor; alveolar rhabdomyosarcoma; embryonal rhabdomyosarcoma; benign or malignant schwannoma; synovial sarcoma; Evan's tumor; nodular fasciitis; desmoid-type fibromatosis;
 20 solitary fibrous tumor; dermatofibrosarcoma protuberans (DFSP); angiosarcoma; epithelioid hemangioendothelioma; tenosynovial giant cell tumor (TGCT); pigmented villonodular synovitis (PVNS); fibrous dysplasia; myxofibrosarcoma; fibrosarcoma; synovial sarcoma; malignant peripheral nerve sheath tumor; neurofibroma; and pleomorphic adenoma of soft tissue; and neoplasias derived from fibroblasts, myofibroblasts, histiocytes, vascular cells/endothelial cells
 25 and nerve sheath cells.

A sarcoma is a rare type of cancer that arises in cells of mesenchymal origin, e.g., in bone or in the soft tissues of the body, including cartilage, fat, muscle, blood vessels, fibrous tissue, or other connective or supportive tissue. Different types of sarcoma are based on where the cancer forms. For example, osteosarcoma forms in bone, liposarcoma forms in fat, and
 30 rhabdomyosarcoma forms in muscle. Examples of sarcomas include, but are not limited to: skin's tumor; sarcoma botryoides; chondrosarcoma; ewing's sarcoma; malignant hemangioendothelioma; malignant schwannoma; osteosarcoma; and soft tissue sarcomas (e.g., alveolar soft part sarcoma; angiosarcoma; cystosarcoma phyllodes; dermatofibrosarcoma protuberans (DFSP); desmoid tumor; desmoplastic small round cell tumor; epithelioid sarcoma;

extraskelatal chondrosarcoma; extraskelatal osteosarcoma; fibrosarcoma; gastrointestinal stromal tumor (GIST); hemangiopericytoma; hemangiosarcoma (more commonly referred to as "angiosarcoma"); kaposi's sarcoma; leiomyosarcoma; liposarcoma; lymphangiosarcoma; malignant peripheral nerve sheath tumor (MPNST); neurofibrosarcoma; synovial sarcoma; undifferentiated pleomorphic sarcoma, and the like).

A teratomas is a type of germ cell tumor that may contain several different types of tissue (e.g., can include tissues derived from any and/or all of the three germ layers: endoderm, mesoderm, and ectoderm), including for example, hair, muscle, and bone. Teratomas occur most often in the ovaries in women, the testicles in men, and the tailbone in children.

Melanoma is a form of cancer that begins in melanocytes (cells that make the pigment melanin). It may begin in a mole (skin melanoma), but can also begin in other pigmented tissues, such as in the eye or in the intestines.

Leukemias are cancers that start in blood-forming tissue, such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the bloodstream. For example, leukemias can originate in bone marrow-derived cells that normally mature in the bloodstream. Leukemias are named for how quickly the disease develops and progresses (e.g., acute versus chronic) and for the type of white blood cell that is effected (e.g., myeloid versus lymphoid). Myeloid leukemias are also called myelogenous or myeloblastic leukemias. Lymphoid leukemias are also called lymphoblastic or lymphocytic leukemia. Lymphoid leukemia cells may collect in the lymph nodes, which can become swollen. Examples of leukemias include, but are not limited to: Acute myeloid leukemia (AML), Acute lymphoblastic leukemia (ALL), Chronic myeloid leukemia (CML), and Chronic lymphocytic leukemia (CLL).

Lymphomas are cancers that begin in cells of the immune system. For example, lymphomas can originate in bone marrow-derived cells that normally mature in the lymphatic system. There are two basic categories of lymphomas. One kind is Hodgkin lymphoma (HL), which is marked by the presence of a type of cell called the Reed-Sternberg cell. There are currently 6 recognized types of HL. Examples of Hodgkin lymphomas include: nodular sclerosis classical Hodgkin lymphoma (CHL), mixed cellularity CHL, lymphocyte-depletion CHL, lymphocyte-rich CHL, and nodular lymphocyte predominant HL.

The other category of lymphoma is non-Hodgkin lymphomas (NHL), which includes a large, diverse group of cancers of immune system cells. Non-Hodgkin lymphomas can be further divided into cancers that have an indolent (slow-growing) course and those that have an aggressive (fast-growing) course. There are currently 61 recognized types of NHL. Examples of non-Hodgkin lymphomas include, but are not limited to: AIDS-related Lymphomas, anaplastic

large-cell lymphoma, angioimmunoblastic lymphoma, blastic NK-cell lymphoma, Burkitt's lymphoma, Burkitt-like lymphoma (small non-cleaved cell lymphoma), chronic lymphocytic leukemia/small lymphocytic lymphoma, cutaneous T-Cell lymphoma, diffuse large B-Cell lymphoma, enteropathy-type T-Cell lymphoma, follicular lymphoma, hepatosplenic gamma-delta
5 T-Cell lymphomas, T-Cell leukemias, lymphoblastic lymphoma, mantle cell lymphoma, marginal zone lymphoma, nasal T-Cell lymphoma, pediatric lymphoma, peripheral T-Cell lymphomas, primary central nervous system lymphoma, transformed lymphomas, treatment-related T-Cell lymphomas, and Waldenstrom's macroglobulinemia.

Brain cancers include any cancer of the brain tissues. Examples of brain cancers
10 include, but are not limited to: gliomas (e.g., glioblastomas, astrocytomas, oligodendrogliomas, ependymomas, and the like), meningiomas, pituitary adenomas, vestibular schwannomas, primitive neuroectodermal tumors (medulloblastomas), etc.

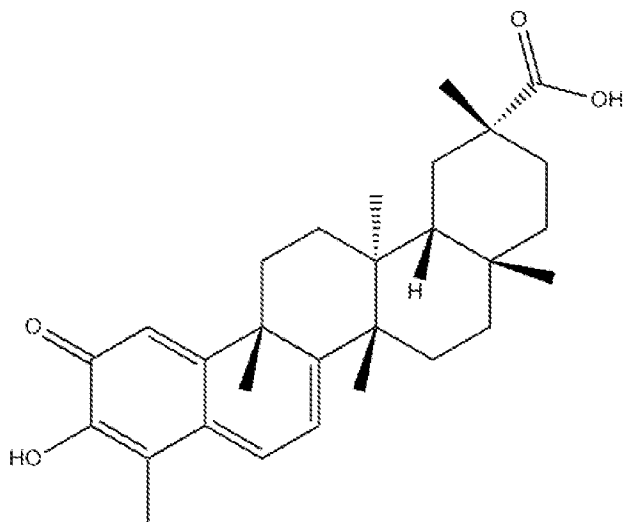
Representative Embodiments

15 The following substituents and values are intended to provide representative examples of various aspects and embodiments. These representative values are intended to further define and illustrate such aspects and embodiments and are not intended to exclude other embodiments or to limit the scope of the present disclosure. In this regard, the representation that a particular value or substituent is preferred is not intended in any way to exclude other
20 values or substituents from the present disclosure unless specifically indicated.

These compounds may contain one or more chiral centers and therefore, the
embodiments are directed to racemic mixtures; pure stereoisomers (i.e., enantiomers or diastereomers); stereoisomer-enriched mixtures and the like unless otherwise indicated. When
25 a particular stereoisomer is shown or named herein, it will be understood by those skilled in the art that minor amounts of other stereoisomers may be present in the compositions unless otherwise indicated, provided that the desired utility of the composition as a whole is not eliminated by the presence of such other isomers.

The compositions of the present disclosure include compounds 1-7, shown below.
Pharmaceutical compositions and methods of the present disclosure also contemplate
30 compounds 1-7.

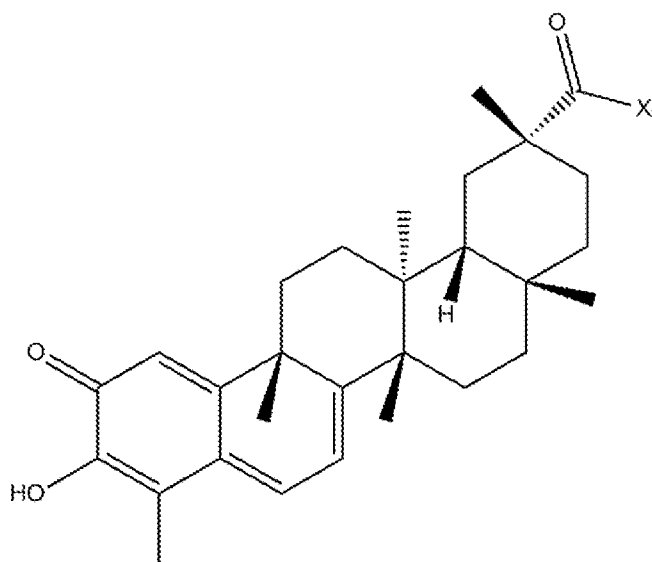
Embodiments of the present disclosure include a compound that is a derivative of (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylic acid (Celastrol):



(Celastrol)

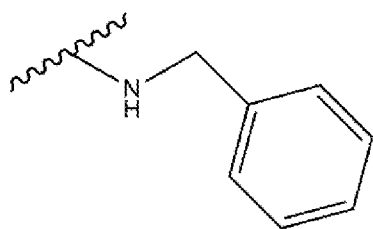
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Embodiments of the present disclosure include a compound of formula (I):

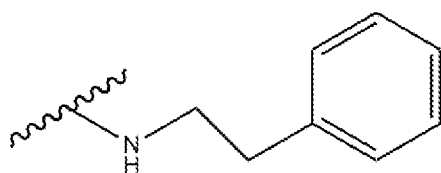


(I)

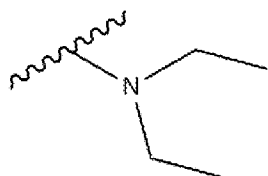
where X is selected from:



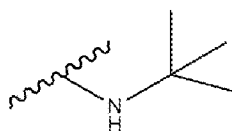
(compound 1; cel-D1);



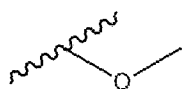
(compound 2; cel-D2);



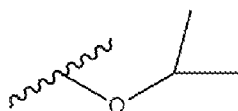
(compound 3; cel-D3)



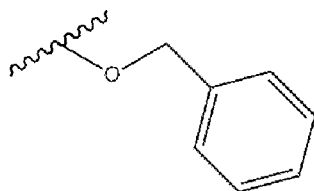
(compound 4; cel-D4);



(compound 5; cel-D5);



(compound 6; cel-D6); and

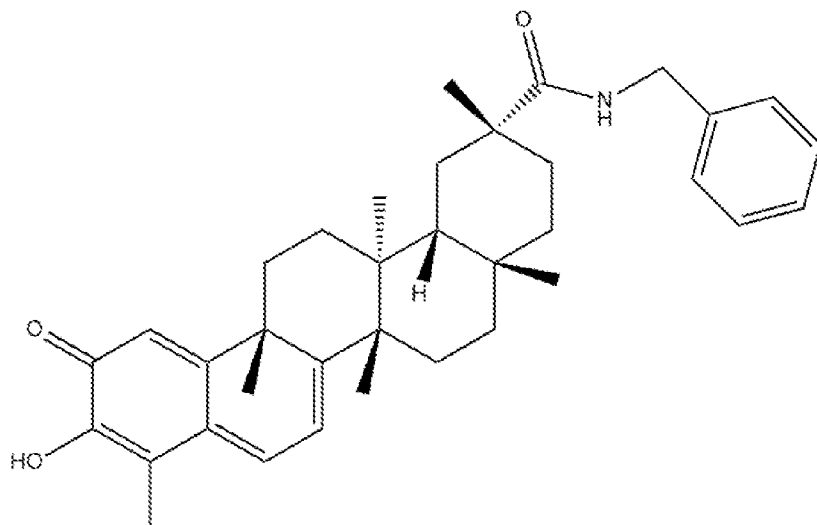


(compound 7; cel-D7);

or a salt or solvate or stereoisomer thereof.

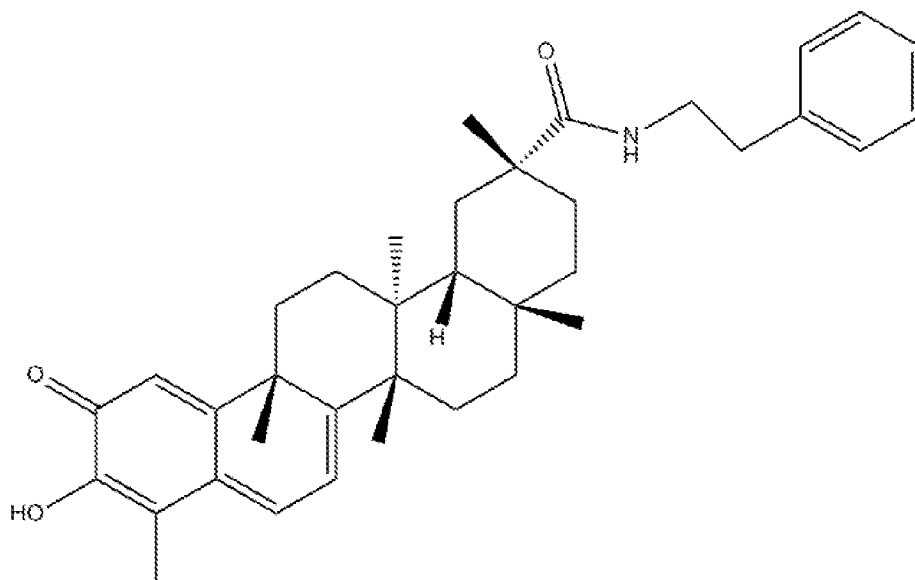
Therefore, particular compounds of interest, and salts or solvates or stereoisomers thereof, include:

- 5 Compound 1 (cel-D1): (2R,4aS,6aR,6aS,14aS,14bR)-N-benzyl-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxamide; also known as: (2R,4aS,6aS,12bR,14aS,14bR)-N-benzyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide:



(Compound 1);

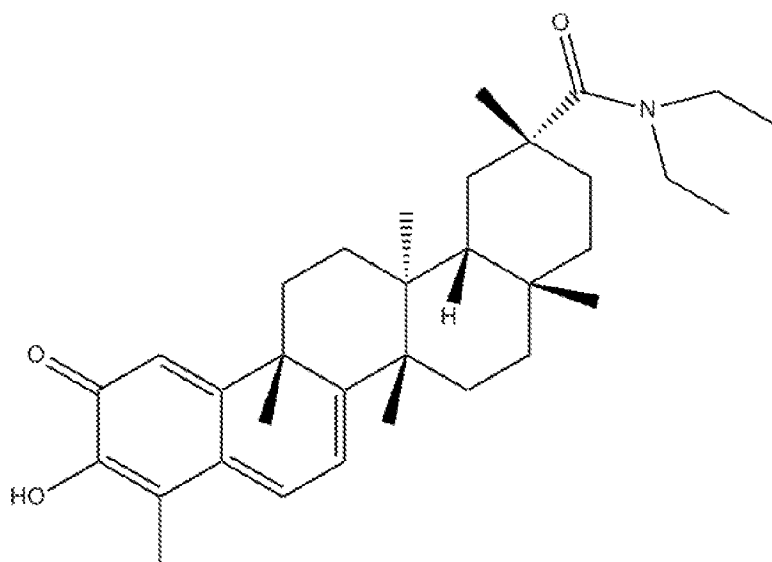
Compound 2 (cel-D2): (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-N-phenethyl-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide:



(Compound 2);

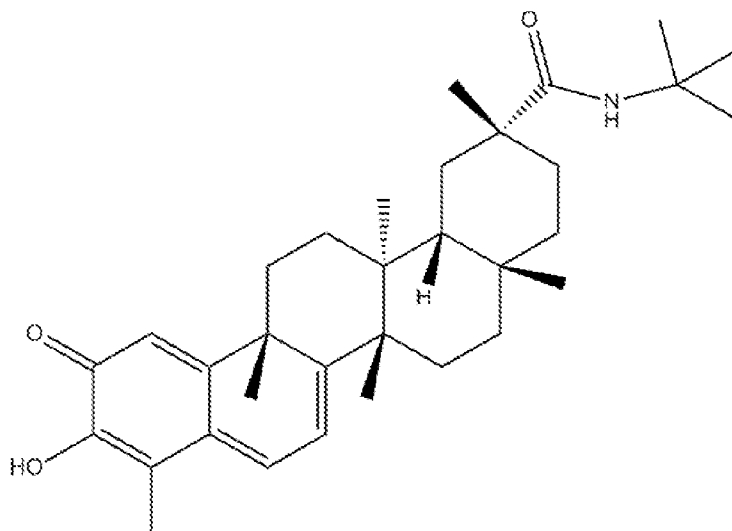
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Compound 3 (cel-D3): (2R,4aS,6aS,12bR,14aS,14bR)-N,N-diethyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide:



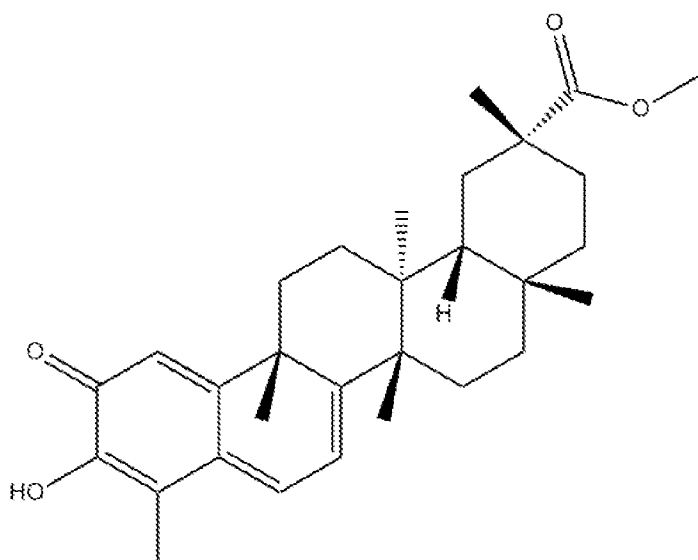
(Compound 3);

Compound 4 (cel-D4): (2R,4aS,6aS,12bR,14aS,14bR)-N-tert-butyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydropicene-2-carboxamide:



5 (Compound 4);

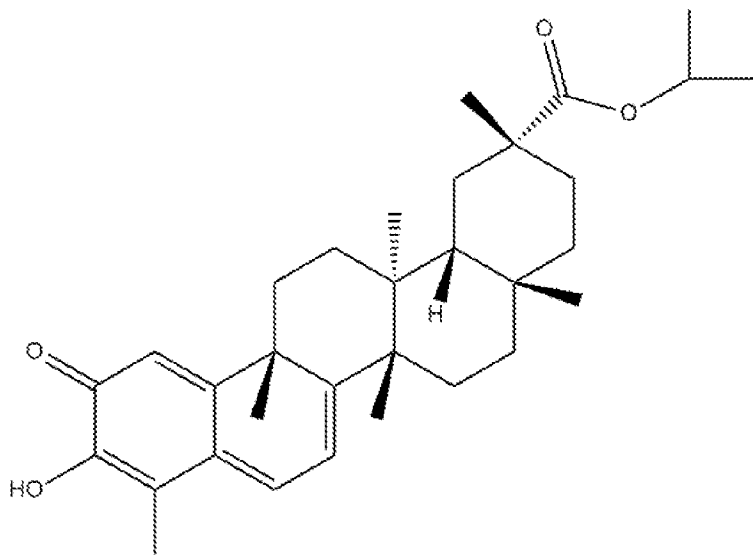
Compound 5 (cel-D5; prestimerin): methyl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydropicene-2-carboxylate:



(Compound 5);

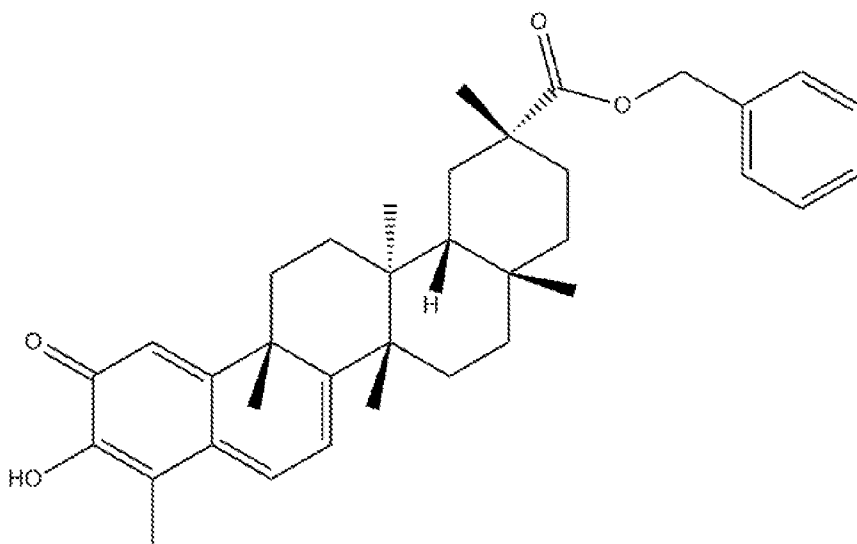
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Compound 6 (cel-D6): propan-2-yl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylate:



(Compound 6); and

5 Compound 7 (cel-D7): benzyl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylate:



(Compound 7).

10 The compounds described also include isotopically labeled compounds where one or more atoms have an atomic mass different from the atomic mass conventionally found in nature. Examples of isotopes that may be incorporated into the compounds disclosed herein include, but are not limited to, ^2H , ^3H , ^{11}C , ^{13}C , ^{14}C , ^{15}N , ^{18}O , ^{17}O , etc. Thus, the subject

compounds may be enriched in one or more of these isotopes relative to the natural abundance of such isotope. By way of example, deuterium (^2H) has a natural abundance of about 0.015%. Accordingly, for approximately every 6,500 hydrogen atoms occurring in nature, there is one deuterium atom. Specifically contemplated herein are compounds enriched in deuterium at one or more positions. Thus, deuterium containing compounds of the disclosure have deuterium at one or more positions (as the case may be) in an abundance of greater than 0.015%.

The present disclosure also provides pharmaceutical compositions that include a pharmaceutically acceptable carrier and a therapeutically effective amount of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, and/or Compound 7; or a pharmaceutically acceptable salt or solvate or stereoisomer thereof.

A subject compound can be administered alone, as the sole active pharmaceutical agent, or in combination with one or more additional subject compounds (e.g., any of Compounds 1-7) or in conjunction with other agents (e.g., in combination with an agent that is a standard of care for a given cancer such as hepatocellular carcinoma, e.g., Sorafenib; Linifanib; sunitinib; an HGF/c-MET inhibitor; an mTOR inhibitor, e.g., an mTOR inhibitor; and FGFR inhibitor; brivanib; tivantinib; cabozantinib; a CTLA-4 blocking compound such as an antibody such as tremelimumab; a PD-1 inhibitor, e.g., BMS-936558; an inhibitor of the Raf/MEK/ERK signaling pathway; an inhibitor of the PI3K/AKT pathway; an inhibitor of the EGFR pathway; temsirolimus; rigosertib; imatinib; dasatinib; nilotinib; and the like; or, for example, in combination with Celastrol). When administered as a combination, the therapeutic agents can be formulated as separate compositions that are administered simultaneously or at different times, or the therapeutic agents can be administered together as a single composition combining two or more therapeutic agents. Thus, the pharmaceutical compositions disclosed herein containing a subject compound optionally include other therapeutic agents. Accordingly, certain embodiments are directed to such pharmaceutical compositions, where the composition further includes a therapeutically effective amount of an agent selected as is known to those of skill in the art.

The subject compounds can antagonize the interaction of HSP90 with CDC37. Accordingly, the compounds are useful for antagonizing the interaction of HSP90 with CDC37. For example, the compounds are useful for treating a disease or disorder (e.g., cancer such as a carcinoma such as hepatocellular carcinoma) that is mediated through the interaction of HSP90 with CDC37 in an individual (e.g., an individual having cancer, e.g., hepatocellular carcinoma). The present disclosure provides a method of antagonizing the interaction of HSP90 with CDC37 (e.g., in a cell, in a cancer cell, in a biological sample, in an individual, in a cell of an

individual, in a cancer cell of an individual, and the like). The present disclosure provides a method of treating an individual having a disease or disorder that is mediated through the interaction of HSP90 with CDC37 in the individual. The present disclosure provides a method of treating an individual having a cell proliferative disorder (e.g., cancer). The present disclosure also provides a method of treating in individual having cancer (e.g., hepatocellular carcinoma). As described above, the term "cancer" includes any form of cancer, including but not limited to solid tumor cancers (e.g., lung, prostate, breast, bladder, colon, ovarian, pancreas, kidney, liver, glioblastoma, medulloblastoma, leiomyosarcoma, head & neck squamous cell carcinomas, melanomas, neuroendocrine; etc.) and liquid cancers (e.g., hematological cancers); carcinomas (e.g., hepatocellular carcinoma); soft tissue tumors; sarcomas; teratomas; melanomas; leukemias; lymphomas; and brain cancers, including minimal residual disease, and including both primary and metastatic tumors.

Since embodiments of the subject compounds possess antagonistic properties toward the interaction of HSP90 with CDC37, such compounds are also useful as research tools.

Accordingly, the disclosure also provides for a method for using a subject compound (e.g., any of compounds 1-7), a salt or solvate or stereoisomer thereof, as a research tool for studying a biological system or samples, or for discovering new chemical compounds having antagonistic properties toward the interaction of HSP90 and CDC37.

The embodiments are also directed to a subject compound (Compounds 1-7) or a salt or solvate or stereoisomer thereof, for use in therapy or as a medicament.

Additionally, the embodiments are directed to the use of a subject compound or a salt or solvate or stereoisomer thereof, for the manufacture of a medicament; for example, for the manufacture of a medicament for antagonizing the interaction of HSP90 and CDC37. The embodiments are also directed to the use of a subject compound or a salt or solvate or stereoisomer thereof for the manufacture of a medicament for the treatment of a disease or disorder mediated or sustained through the interaction of HSP90 with CDC37. The embodiments are also directed to the use of a subject compound or a salt or solvate or stereoisomer thereof for the manufacture of a medicament for the treatment of cancer (e.g., a carcinoma such as HCC). The embodiments of the present disclosure are also directed to the use of a subject compound or a salt or solvate or stereoisomer thereof for the manufacture of a medicament for the treatment of a cell proliferative disorder.

General Synthetic Procedures

Many general references providing commonly known chemical synthetic schemes and conditions useful for synthesizing the subject compounds are available (see, e.g., Smith and March, March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, Fifth Edition, Wiley-Interscience, 2001; or Vogel, A Textbook of Practical Organic Chemistry, Including Qualitative Organic Analysis, Fourth Edition, New York: Longman, 1978).

Compounds as described herein can be purified by any purification protocol known in the art, including chromatography, such as HPLC, preparative thin layer chromatography, flash column chromatography and ion exchange chromatography. Any suitable stationary phase can be used, including normal and reversed phases as well as ionic resins. In certain embodiments, the subject compounds are purified via silica gel and/or alumina chromatography. See, e.g., Introduction to Modern Liquid Chromatography, 2nd Edition, ed. L. R. Snyder and J. J. Kirkland, John Wiley and Sons, 1979; and Thin Layer Chromatography, ed E. Stahl, Springer-Verlag, New York, 1969.

During any of the processes for preparation of the subject compounds, it may be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules concerned. This may be achieved by means of conventional protecting groups as described in standard works, such as J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London and New York 1973, in T. W. Greene and P. G. M. Wuts, "Protective Groups in Organic Synthesis", Third edition, Wiley, New York 1999, in "The Peptides"; Volume 3 (editors: E. Gross and J. Meienhofer), Academic Press, London and New York 1981, in "Methoden der organischen Chemie", Houben-Weyl, 4th edition, Vol. 15/I, Georg Thieme Verlag, Stuttgart 1974, in H.-D. Jakubke and H. Jescheit, "Aminosäuren, Peptide, Proteine", Verlag Chemie, Weinheim, Deerfield Beach, and Basel 1982, and/or in Jochen Lehmann, "Chemie der Kohlenhydrate: Monosaccharide and Derivate", Georg Thieme Verlag, Stuttgart 1974. The protecting groups may be removed at a convenient subsequent stage using methods known from the art.

Synthesis of Compounds

The subject compounds can be synthesized via a variety of different synthetic routes using commercially available starting materials and/or starting materials prepared by conventional synthetic methods. Suitable examples of methods that can be adapted to synthesize the subject compounds disclosed herein are found in U.S. Patent Nos. 7,888,355; and 7,776,894, the disclosures of which are hereby incorporated by reference.

In certain embodiments, the amide Celstrol derivatives and the ester Celstrol derivatives

of the subject compounds can be synthesized by contacting (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylic acid (Celastrol) with an appropriate amine (e.g., benzylamine (1-phenylmethanamine) for cel-D1, phenethylamine (2-phenylethylamine) for cel-D2; diethylamine (ethanamine) for cel-D3; or
5 tert-butylamine (2-propanamine) for cel-D4) or appropriate alkyl halide (e.g. 2-bromopropane for cel-D6, benzyl bromide for cel-D7).

In some cases (e.g., when synthesizing an amide Celstrol derivative, e.g., Compounds 1-4), the compounds react in a polar aprotic solvent or a polar protic solvent. In some cases, the compounds react in a polar aprotic solvent. In some cases, the compounds react in a polar
10 protic solvent. Suitable polar aprotic solvents can include dimethylformamide, tetrahydrofuran, dimethylsulfoxide, dioxane, and the like. Suitable polar protic solvents can include alcohols (e.g., isopropanol, methanol, ethanol, etc.), formic acid, and the like. In some embodiments, the reaction is carried out in the presence of a base. In some cases, the base can be a poor nucleophile (e.g., Diisopropylethylamine). In some embodiments, the reaction is carried out in
15 the presence of a coupling reagent. In some cases, the coupling reagent is (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP). In some cases, a method of synthesizing a subject compound includes contacting (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylic acid (Celastrol) with benzylamine (1-phenylmethanamine) (e.g. to synthesize Compound 1),
20 phenethylamine (2-phenylethylamine)(e.g., to synthesize Compound 2), diethylamine (ethanamine) (e.g., to synthesize Compound 3), or tert-butylamine (2-propanamine) (e.g., to synthesize Compound 4). The reaction can be run at room temperature or can be heated.

In some cases (e.g., when synthesizing an ester Celstrol derivative, e.g., Compounds 5-7), the compounds react in a polar aprotic solvent or a polar protic solvent. In some cases, the
25 compounds react in a polar aprotic solvent. In some cases, the compounds react in a polar protic solvent. Suitable polar aprotic solvents can include dimethylformamide, tetrahydrofuran, dimethylsulfoxide, dioxane, and the like. Suitable polar protic solvents can include alcohols (e.g., isopropanol, methanol, ethanol, etc.), formic acid, and the like. In some embodiments, the reaction is carried out in the presence of sodium bicarbonate. The reaction can be run at room
30 temperature or can be heated.

In certain embodiments, in the above methods, the method further includes separating isomers with a resolution technique. In certain embodiments, in the above methods, the method further includes separating isomers with chiral chromatography.

In some embodiments, the above methods further include the step of forming a salt of a

subject compound. Embodiments are directed to the other processes described herein, and to the product prepared by any of the processes described herein.

In some embodiments, deionized water can be added to the reaction mixture. In some cases, the mixture is extracted (e.g., by ethyl acetate). The combined organic extracts can be dried (e.g., over $MgSO_4$), and evaporated, filtered, and concentrated (e.g., via a rotary evaporator), which in some cases will yield a dark red oil. The product can be purified (e.g., by flash chromatography (ethyl acetate:hexanes); reversed phase (RP)-HPLC, e.g., using a C18 column with an acetonitrile-water gradient mobile phase) and lyophilized, which in some cases will result in a dark orange to red solid.

Pharmaceutical Compositions

In certain embodiments, the disclosed compounds are useful for the inhibition of PKC activity and the treatment of a disease or disorder that is mediated through the activity of a PKC activity. Accordingly, pharmaceutical compositions comprising at least one disclosed compound are also described herein.

A pharmaceutical composition that includes a subject compound may be administered to a patient alone, or in combination with other supplementary active agents. The pharmaceutical compositions may be manufactured using any of a variety of processes, including, but not limited to, conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, lyophilizing, and the like. The pharmaceutical composition can take any of a variety of forms including, but not limited to, a sterile solution, suspension, emulsion, lyophilisate, tablet, pill, pellet, capsule, powder, syrup, elixir or any other dosage form suitable for administration.

A subject compound may be administered to a subject using any convenient means capable of resulting in the desired reduction in disease condition or symptom. Thus, a subject compound can be incorporated into a variety of formulations for therapeutic administration. More particularly, a subject compound can be formulated into pharmaceutical compositions by combination with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, aerosols, and the like.

Formulations for pharmaceutical compositions are described in, for example, Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, Pa., 19th Edition, 1995, which describes examples of formulations (and components thereof) suitable for

pharmaceutical delivery of disclosed compounds. Pharmaceutical compositions that include at least one of the subject compounds can be formulated for use in human or veterinary medicine. Particular formulations of a disclosed pharmaceutical composition may depend, for example, on the mode of administration and/or on the location of the subject to be treated. In some
5 embodiments, formulations include a pharmaceutically acceptable carrier in addition to at least one active ingredient, such as a subject compound. In other embodiments, other medicinal or pharmaceutical agents, for example, with similar, related or complementary effects on the disease or condition being treated can also be included as active ingredients in a pharmaceutical composition.

10 Pharmaceutically acceptable carriers useful for the disclosed methods and compositions may depend on the particular mode of administration being employed. For example, parenteral formulations may include injectable fluids, such as, but not limited to, pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (e.g., powder, pill,
15 tablet, or capsule forms), non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered can optionally contain minor amounts of non-toxic auxiliary substances (e.g., excipients), such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like; for example, sodium acetate or sorbitan
20 monolaurate. Other examples of excipients include, nonionic solubilizers, such as cremophor, or proteins, such as human serum albumin or plasma preparations.

Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl
25 cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol, and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium
30 hydroxide and aluminum hydroxide; (15) alginic acid; (16) water (e.g., pyrogen-free water); (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

The disclosed pharmaceutical compositions may be formulated as a pharmaceutically

acceptable salt of a disclosed compound. Pharmaceutically acceptable salts are non-toxic salts of a free base form of a compound that possesses the desired pharmacological activity of the free base. These salts may be derived from inorganic or organic acids. Non-limiting examples of suitable inorganic acids are hydrochloric acid, nitric acid, hydrobromic acid, sulfuric acid, hydroiodic acid, and phosphoric acid. Non-limiting examples of suitable organic acids are acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, methyl sulfonic acid, salicylic acid, formic acid, trichloroacetic acid, trifluoroacetic acid, gluconic acid, asparagic acid, aspartic acid, benzenesulfonic acid, *para*-toluenesulfonic acid, naphthalenesulfonic acid, and the like. In certain embodiments, the pharmaceutically acceptable salt includes formic acid. In certain embodiments, the pharmaceutically acceptable salt includes trifluoroacetic acid. Other suitable pharmaceutically acceptable salts are found in Remington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Company, Easton, Pa., 1985. A pharmaceutically acceptable salt may also serve to adjust the osmotic pressure of the composition.

A subject compound can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents. Such preparations can be used for oral administration.

A subject compound can be formulated into preparations for injection by dissolving, suspending or emulsifying the compound in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles. Formulations suitable for injection can be administered by an intravitreal, intraocular, intramuscular, subcutaneous, sublingual, or other route of administration, e.g., injection into the gum tissue or other oral tissue. Such formulations are also suitable for topical administration.

In some embodiments, a subject compound can be delivered by a continuous delivery system. The term "continuous delivery system" is used interchangeably herein with "controlled delivery system" and encompasses continuous (e.g., controlled) delivery devices (e.g., pumps)

in combination with catheters, injection devices, and the like, a wide variety of which are known in the art.

A subject compound can be utilized in aerosol formulation to be administered via inhalation. A subject compound can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

Furthermore, a subject compound can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. A subject compound can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are substantially solid at room temperature.

The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of a subject compound calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for a subject compound depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

The dosage form of a disclosed pharmaceutical composition may be determined by the mode of administration chosen. For example, in addition to injectable fluids, topical or oral dosage forms may be employed. Topical preparations may include eye drops, ointments, sprays and the like. Oral formulations may be liquid (e.g., syrups, solutions or suspensions), or solid (e.g., powders, pills, tablets, or capsules). Methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art.

Certain embodiments of the pharmaceutical compositions that include a subject compound may be formulated in unit dosage form suitable for individual administration of precise dosages. The amount of active ingredient administered may depend on the subject being treated, the severity of the affliction, and the manner of administration, and is known to those skilled in the art. In certain instances, the formulation to be administered contains a quantity of the compounds disclosed herein in an amount effective to achieve the desired effect in the subject being treated.

Each therapeutic compound can independently be in any dosage form, such as those described herein, and can also be administered in various ways, as described herein. For example, the compounds may be formulated together, in a single dosage unit (that is, combined together in one form such as capsule, tablet, powder, or liquid, etc.) as a combination product. Alternatively, when not formulated together in a single dosage unit, an individual subject

compound may be administered at the same time as another therapeutic compound or sequentially, in any order thereof.

Methods of Administration

5 The subject compounds can antagonize the interaction of HSP90 with CDC37. Accordingly, the compounds are useful for antagonizing the interaction of HSP90 with CDC37. For example, the compounds are useful for treating a disease or disorder (e.g., cancer such as a carcinoma such as hepatocellular carcinoma) that is mediated through the interaction of HSP90 with CDC37 in an individual (e.g., an individual having cancer, e.g., hepatocellular carcinoma). The present disclosure provides a method of antagonizing the interaction of HSP90 with CDC37 (e.g., in a cell, in a cancer cell, in a biological sample, in an individual, in a cell of an individual, in a cancer cell of an individual, and the like). The present disclosure provides a method of treating an individual having a disease or disorder that is mediated through the interaction of HSP90 with CDC37 in the individual. The present disclosure provides a method of
10 treating an individual having a cell proliferative disorder (e.g., cancer). The present disclosure also provides a method of treating in individual having cancer (e.g., hepatocellular carcinoma).

 The route of administration of a subject compound (e.g., any of Compounds 1-7) may be selected according to a variety of factors including, but not limited to, the condition to be treated, the formulation and/or device used, the patient to be treated, and the like. Routes of
20 administration useful in the disclosed methods include but are not limited to oral and parenteral routes, such as intravenous (iv), intraperitoneal (ip), rectal, topical, ophthalmic, nasal, and transdermal. Formulations for these dosage forms are described herein.

 An effective amount of a subject compound may depend, at least, on the particular method of use, the subject being treated, the severity of the affliction, and the manner of
25 administration of the therapeutic composition. A “therapeutically effective amount” of a composition is a quantity of a specified compound sufficient to achieve a desired effect in an individual (e.g., patient) being treated. For example, this may be the amount of a subject compound necessary to prevent, inhibit, reduce or relieve a disease or disorder that is mediated through the interaction of HSP90 and CDC37 (e.g., hepatocellular carcinoma). Ideally, a
30 therapeutically effective amount of a compound is an amount sufficient to prevent, inhibit, reduce or relieve a disease or disorder that is mediated through the interaction of HSP90 and CDC37 in an individual without causing a substantial cytotoxic effect on host cells (e.g., non-cancerous cells of the individual).

Therapeutically effective doses of a subject compound or pharmaceutical composition can be determined by one of skill in the art, with a goal of achieving local (e.g., tissue) concentrations that are at least as high as the IC_{50} of an applicable compound disclosed herein.

An example of a dosage range is from 0.1 to 200 mg/kg body weight orally in single or
5 divided doses. In some embodiments, a dosage range is from 1.0 to 100 mg/kg body weight orally in single or divided doses, including from 1.0 to 50 mg/kg body weight, from 1.0 to 25 mg/kg body weight, from 1.0 to 10 mg/kg body weight, from 0.5 to 25 mg/kg body weight, from 0.5 to 10 mg/kg body weight, from 5.0 to 25 mg/kg body weight, and/or from 5.0 to 10 mg/kg body weight (assuming an average body weight of approximately 70 kg; values may be adjusted
10 accordingly for persons weighing more or less than average). For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 10 to about 1000 mg of the active ingredient, such as 25 to 750 mg, or 50 to 500 mg, for example 75 mg, 100 mg, 200 mg, 250 mg, 400 mg, 500 mg, 600 mg, 750 mg, or 1000 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject being treated. In certain
15 embodiments of an oral dosage regimen, a tablet containing from 500 mg to 1000 mg active ingredient is administered once (e.g., a loading dose) followed by administration of 1/2 (i.e., half) dosage tablets (e.g., from 250 to 500 mg) each 6 to 24 hours for 3 days or more.

The specific dose level and frequency of dosage for any particular subject may be varied and may depend upon a variety of factors, including the activity of the subject compound, the
20 metabolic stability and length of action of that compound, the age, body weight, general health, sex and diet of the subject, mode and time of administration, rate of excretion, drug combination, and severity of the condition of the host undergoing therapy.

Embodiments of the present disclosure also include combinations of one or more disclosed compounds with one or more other agents or therapies useful in the treatment of a
25 disease or disorder. In certain instances, the disease or disorder is mediated through the interaction of HSP90 with CDC37. In certain instances, the disease or disorder is cell proliferative disorder (e.g., cancer). For example, one or more disclosed compounds may be administered in combination with therapeutically effective doses of other medicinal and pharmaceutical agents, or in combination other non-medicinal therapies, such as hormone or
30 radiation therapy. The term "administration in combination with" refers to both concurrent and sequential administration of the active agents.

In some cases a subject method can include a step to determine whether the interaction of HSP90 with CDC37 is antagonized (i.e., whether the interaction is reduced). Antagonizing the interaction of HSP90 with CDC37 can be measured by monitoring the ability of HSP90 to

specifically bind to CDC37 in a cell. For example, cell lysates can be prepared from cells that are contacted with a test Compound and a control compound (or no compound at all). Immunoprecipitation can be performed using an antibody against CDC37, and then one can assay for the presence of HSP90 (e.g., via Western blot, mass spectrometry, etc.). Alternatively, immunoprecipitation can be performed using an antibody against HSP90, and then one can assay for the presence of CDC37 (e.g., via Western blot, mass spectrometry, etc.). Any method for detecting whether HSP90 forms a complex with (i.e., interacts with) CDC37 can be used. When less of an interaction is detected in the presence of a test compound compared to a control (e.g., a vehicle only control, or a compound known not to antagonize the interaction), then the test compound can be said to antagonize the interaction between HSP90 and CDC37. In some cases, the compound reduces the interaction to 90% or less (e.g., 80% or less, 70% or less, 60% or less, 50% or less, 40% or less, 30% or less, 20% or less, 10% or less, or 5% or less) compared to control. For example, when immunoprecipitating with an antibody against CDC37 and detecting HSP90, if the sample from the cells contacted with the test compound contains 90% the amount of HSP90 detected in the control sample, the compound can be said to antagonize the interaction of HSP90 with CDC37 (e.g., reduce the interaction to 90% compared to control).

In some cases (e.g., when the method is a method that results in the reduction of the number of cancer cells in an individual, a method of treating an individual having cancer, etc.), the efficacy of a subject compound can be tested by monitoring the presence of cancer (e.g., monitoring the presence of cancer cells, tumors, etc.) in the individual (e.g., following administration of a subject compound).

HSP90 and CDC37

Heat Shock Protein 90kDa alpha, class A member 1 (HSP90AA1) and Heat Shock Protein 90kDa alpha, class B member 1 (HSP90AB1) are sometimes referred to as HSP90 alpha (HSP90 α) and HSP90 beta (HSP90 β), and sometimes referred to collectively as HSP90. Many antibodies used to detect HSP90 detect both proteins. A series of proteins including HSP90, HSP70, HSP20-30, and ubiquitin are induced by insults such as temperature shock, chemicals and other environmental stress. A major function of HSP90 and other heat shock proteins (HSPs) is to act as molecular chaperones. HSP 90 functions as a homodimer. The encoded protein aids in the proper folding of specific target proteins by use of an ATPase activity that is modulated by co-chaperones.

Cell division cycle 37 (CDC37) is highly similar to Cdc37, a cell division cycle control

protein of the yeast *Sacchromyces cerevisiae*. This protein is a molecular co-chaperone with specific function in cell signal transduction. It has been shown to form complex with Hsp90 and a variety of protein kinases including CDK4, CDK6, SRC, RAF-1, MOK, as well as eIF2 alpha kinases. It is thought to play a critical role in directing Hsp90 to its target kinases. For example, CDC 37 is over-expressed in hepatocellular carcinoma (HCC) cells, where it functions with HSP90 to regulate the activity of protein kinases in multiple oncogenic signaling pathways that contribute towards hepatocarcinogenesis.

In another embodiment of the disclosure, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). An active agent in the composition can be any of the subject compounds (Compounds 1-7). The label on, or associated with, the container can indicate that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made without departing from the spirit or scope of the invention.

EXPERIMENTAL

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.* amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for.

Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and
5 individually indicated to be incorporated by reference.

The present invention has been described in terms of particular embodiments found or proposed by the present inventor to comprise preferred modes for the practice of the invention. It will be appreciated by those of skill in the art that, in light of the present disclosure, numerous modifications and changes can be made in the particular embodiments exemplified without
10 departing from the intended scope of the invention. For example, due to codon redundancy, changes can be made in the underlying DNA sequence without affecting the protein sequence. Moreover, due to biological functional equivalency considerations, changes can be made in protein structure without affecting the biological action in kind or amount. All such modifications are intended to be included within the scope of the appended claims.

Example 1

We hypothesized that disrupting the HSP90 and CDC37 chaperone complex would achieve anti-tumor effects in HCC, by inducing degradation and inhibiting phosphorylation of their client proteins kinases. The recently identified HSP90/CDC37 antagonist, celastrol, was
20 evaluated for its anti-tumor activity in HCC cell lines and patient-derived xenografts. In addition, derivatives of celastrol were synthesized and compared regarding safety and anti-tumor activity profiles in HCC patient-derived xenografts, a clinically relevant model for evaluating the performance of these compounds.

Materials and methods

Synthesis of celastrol derivatives

Celastrol and pristimerin were purchased from Sigma-Aldrich (St. Louis, MO). Two other celastrol derivatives, cel-D2 and cel-D7, were synthesized using celastrol as the starting material, using chemicals purchased from Sigma-Aldrich. Synthetic schemes for cel-D2 and cel-
30 D7 are shown in Figure 9, and are exemplary for the synthesis of the subject compounds (amide derivatives: compounds 1-4; and ester derivatives: compounds 5-7). The ¹H and ¹³C NMR spectra were obtained using the Varian 300 MHz or 400 MHz magnetic resonance spectrometer. High resolution mass spectrometric (MS) analyses of the compounds were performed at the Mass Spectrometry Facility at Stanford University. For cel-D2, celastrol (20.5

mg, 0.045 mmol) was dissolved in dimethylformamide (2 mL), DIPEA (20 μ L, 0.12 mmol) and PyBOP (50 mg, 0.096 mmol) were then added into the solution, followed by 2-phenylethylamine (10 μ L, 0.079 mmol). After stirring for 24 hours at room temperature, deionized water (15 mL) was added and the reaction mixture was extracted by ethyl acetate (3 X 15 mL). The combined
5 organic extracts were dried over MgSO₄, filtered and concentrated *via* a rotary evaporator to yield a dark red oil. Reversed phase (RP)-HPLC (Dionex HPLC System; Dionex Corporation, Sunnyvale, CA) using a C18 column (Phenomenax, 5 μ m, 4.6 x 250 mm or Dionex, 5 μ m, 21.2 x 250 mm) and an acetonitrile-water gradient mobile phase (flow of 1 or 12 mL/minutes) afforded cel-D2 as an orange solid (17.2 mg, 68.0% yield), with m/z 554.3621 (M+H).

10 For cel-D7, celastrol (20.2 mg, 0.045 mmol) was dissolved in dimethylformamide (2 mL), followed by addition of sodium bicarbonate (21.5 mg, 0.256 mmol) and benzyl bromide (8 μ L, 0.067 mmol). After stirring for 24 hours at room temperature, deionized water (15 mL) was added and the reaction mixture was extracted by ethyl acetate (3 X 15 mL). The combined
15 organic extracts were dried over MgSO₄, filtered and concentrated *via* a rotary evaporator to yield a dark red oil. RP-HPLC purification on a C18 column with an acetonitrile-water gradient afforded cel-D7 as an orange solid (14.0 mg, 57.4% yield), with m/z 541.3306 (M+H).

Detailed characterization data of cel-D2 and cel-D7 are provided in Figs. 10-15.

20 Culture of HCC cell lines and primary hepatocytes

Human HCC cell lines HepG2, Huh7, and Hep3B were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA), 100 μ g/ml penicillin, and 100 μ g/ml streptomycin. Cells were cultured at 37°C
25 in a humidified atmosphere with 5% CO₂. The HepG2 and Hep3B cell lines were obtained from American Type Culture Collection (Manassas, VA) in 2008. The Huh7 cell line was a gift from Dr. Mark Kay (Stanford University, CA) in 2003. All cell lines were last authenticated in June 2013, by the short tandem repeat profiling method at the Johns Hopkins Genetic Resources Core Facility. And all cell lines used were tested regularly for
30 mycoplasma contamination.

Cryopreserved normal human hepatocytes and all special media and plates needed for their culture were received from CellzDirect/Invitrogen (Durham, NC). Characteristics of the three hepatocyte lots are shown in **Table 1**. Thawing and culture of primary hepatocytes were as described previously.

Table 1. Characteristics of normal hepatocytes

Lot	Age (y)	Gender	Race	Cell viability
Hu4175	3	Male	Caucasian	79%
Hu8114	47	Female	Caucasian	95%
Hu8130	18	Female	Caucasian	92%

Protein extraction, Western blotting, and co-immunoprecipitation

Total protein was extracted from tissues or harvested cells using T-PER Tissue Protein Extraction Reagent (Pierce; Rockford, IL), and protein concentration was determined with BCA Protein Assay Kit (Pierce, Rockford, IL). Equal amounts of protein (10 µg) were electrophoresed on 4% to 12% polyacrylamide gels (Invitrogen; Carlsbad, CA), and transferred onto polyvinylidene difluoride membranes, blocked with 10% nonfat milk for at least 1 hour, and probed with primary antibodies against CDC37 (ab61773) from Abcam (Cambridge, MA); HSP90 (SC-59577), B-Raf (SC-166), GAPDH (SC-365062) from Santa Cruz Biotechnology (Santa Cruz, CA); CDK4 (2906), EGFR (2232), MEK1/2 (9122), AKT (9272), A-Raf (4432), C-Raf (9422), p-A-Raf (s299, 4431), p-B-Raf (s445,2696), p-C-Raf (s289/296/301, 9431), p-AKT (s473, 4058s), p-MEK1/2 (s217/221, 9121) from Cell Signaling (Danvers, MA). The specific proteins were detected with HRP-conjugated secondary antibodies (Santa Cruz Biotechnology; Santa Cruz, CA) and SuperSignal West Pico or West Femto Maximum Sensitivity substrate from Pierce (Rockford, IL). Co-immunoprecipitation was performed using Protein A/G agarose (SC-2003, Santa Cruz Biotechnology, Santa Cruz, CA) according to manufacturer's instructions, with anti-CDC37 antibody (ab61773, Abcam, Cambridge, MA) for pull-down, and anti-HSP90 antibody (SC-59577, Santa Cruz Biotechnology, Santa Cruz, CA) for immunoblotting.

Cell viability assay

Test compounds were added at desired final concentrations, and further incubated for 72 hours before cell viability was assessed using CellTiter-Glo Luminescent Cell Viability Assay (Promega; Madison, Wisconsin) as previously described. The 50% inhibitory concentrations (IC₅₀s; concentration of drug that inhibits cell growth by 50%) were calculated as an estimate of the cytotoxic effects of the four compounds. Three independent experiments were done, each in triplicates.

Apoptosis analysis

Terminal dUTP-mediated nick-end labeling (TUNEL) assays (Promega, Madison, WI, USA) were performed according to the manufacturer's protocol. Briefly, HepG2 cells were seeded in 8-chamber BD tissue culture slides (BD Bioscience Labware, Bedmord, MA) at 10%
5 confluency. Celastrol or its derivatives were added to the culture medium at final concentrations of 5 μ M. After 6 hours incubation, cells were washed twice with PBS, and then fixed in 4% paraformaldehyde for 25 minutes. Fixed cells were washed twice in PBS with 0.1% Triton X-100, and then incubated with TUNEL reaction mixture for 60 minutes at 37°C. After washing with 2xSSC, slides were immersed in PBS (with 5 μ g/ml DAPI) for 5 minutes in the dark, and
10 then washed with PBS. Fluorescence labeling was visualized and photographed (100x magnification) with a fluorescence microscope (Nikon Eclipse 80i, Nikon Corporation, Tokyo, Japan) and with a digital camera (Nikon DXM1200f, Nikon Corporation, Tokyo, Japan). For TUNEL staining of the patient-derived xenografts, 6- μ m tissue sections were stained using the ApopTag Peroxidase in Situ Oligo Ligation Apoptosis Detection Kit (Chemicon International,
15 Temecula, CA) according to the manufacturer's protocol.

Establishment of orthotopic HCC patient-derived xenografts

HCC tissues were collected from HCC patients who had undergone liver resection as part of their treatment. This study was approved by the Institutional Review Board at Stanford
20 University for the use of human subjects in medical research, and informed consent was obtained from each patient prior to liver resection. Animal studies were carried out in compliance with Federal and local institutional rules for the conduct of animal experiments.

Characteristics of the three HCC patients are shown in Table 2. HCC specimens were mechanically and enzymatically dissociated in HBSS containing 0.1% collagenase, 0.01%
25 hyaluronidase and 0.002% deoxyribonuclease at 37°C to obtain single cell suspensions. Cells were then passed through a 70- μ m filter, centrifuged at 100 g for 10 minutes and resuspended in Freezing Medium (FBS containing 10% DMSO) for storage at -80°C overnight, and transferred to liquid nitrogen for long-term storage. Thawed cells were suspended in BEGM medium mixed with 50% Matrix Matrigel (Becton Dickinson; Franklin Lakes, NJ) and injected
30 subcutaneously into 4 week old (20 g body weight), male NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (Nod-SCID-Gamma; NSG). Mating pairs of NSG mice were originally purchased from Jackson Laboratory (Bar Harbor, MA), and bred according to approved institutional protocols. Once the subcutaneous xenografts reached 1 cm in diameter, they were harvested for dissociation as described above. Single cell suspensions were then transduced with self-inactivating lentivirus

carrying an ubiquitin promoter driving a trifusion reporter gene, which harbors a bioluminescence (Luc2), a fluorescence (egfp), and a positron emission tomography reporter gene (ttk) at a multiplicity of infection of 5. High titer lentiviral vectors were produced using a modified version of a previous protocol. Tumor cells were stained with Pacific Blue™ anti-mouse CD45, Pacific Blue™ anti-mouse H-2Kd, and Pacific Blue™ anti-mouse CD31 (BioLegend; San Diego, CA). Stable expressors were isolated by sorting as eGFP positive and Pacific Blue negative cells performed on a BD FACS Aria (Becton Dickinson; Franklin Lakes, NJ).

Table 2. Characteristics of HCC patients

	Age (y)	Gender	Race	Virus
HCC-1	44	Male	Asian	HBV
HCC-2	75	Male	Caucasian	None
HCC-3	35	Male	Asian	HBV

To generate orthotopic HCC patient-derived xenografts, single tumor cells labeled with luciferase gene were suspended in BEME medium containing 50% Matrix Matrigel, and then subcutaneously injected into 4–8 week old male NSG mice (20-25 g body weight). Tumor development was monitored daily. Once the subcutaneous xenograft reached 1 cm in diameter, it was removed and cut into 2 mm³ pieces and surgically implanted into the left lobe of the liver of another group of 6 weeks old NSG mice. Tumor growth was monitored once a week using the Xenogen IVIS *in vivo* imaging system (Caliper Life Sciences, Hopkinton, CA). Firefly luciferase imaging was acquired with D-Luc saline solution (150 mg/Kg body weight) *via* intraperitoneal injection.

Animal treatment with celastrol and its derivatives

Treatment with celastrol and its derivatives were initiated within 1 week after transplantation of the orthotopic patient-derived xenografts. Tumor-bearing mice were randomized into groups (n = 5 each) to be intravenously injected with saline only (control); celastrol (4 mg/Kg); pristimerin (1 mg/Kg); cel-D2 (8 mg/Kg); cel-D7 (8 mg/Kg) (each diluted in saline) three times per week. Tumor growth was monitored weekly using the Xenogen IVIS *in vivo* imaging system, and growth curves were plotted using average bioluminescence within

each group. Body weight was also measured weekly. After 3 weeks treatment, the mice were sacrificed and the tumors and normal livers harvested. Tumor size was measured with digital calipers and tumor volume was calculated using the formula $\pi/6 \times \text{larger diameter} \times [\text{smaller diameter}]^2$. Liver and tumor tissues were fixed in formalin and embedded with paraffin. The 6-
5 μm tissue sections were stained with hematoxylin and eosin (H&E) for evaluation of cell morphology and apoptosis as described above.

In vivo toxicity studies of celastrol and its derivatives

BALB/cJ mice (6-8 weeks old) were randomized into groups ($n = 4$ each) to be
10 intravenously injected with saline only (control); celastrol (4 mg/Kg); pristimerin (1 mg/Kg); cel-D2 (8 mg/Kg); or cel-D7 (8 mg/Kg) (each diluted in saline) three times per week. At the end of the administration period, mice in each group were euthanized and their bodies and harvested organs were weighed. In addition, blood was collected for whole blood complete blood counts (CBC) and plasma chemistry analysis at the Stanford Animal Diagnostic Laboratory. The normal
15 ranges of CBC and plasma chemistry panel are obtained from the Mouse Phenome Database of the Jackson Laboratory.

Statistical analysis

Statistical analyses were done using the SPSS version 15.0 software package (SPSS,
20 Inc, Chicago, IL). Statistical significance was determined by independent samples t-test. $P < 0.05$ and $P < 0.01$ were considered statistically significant and highly significant, respectively.

Results

Synthesis and characterization of celastrol derivatives that disrupt HSP90/CDC37 complexes

The chemical structures of celastrol and its derivatives are shown in Table 3. All chemical structures were confirmed by analyses using ^1H (Fig. 10 for cel-D2; Fig. 11 for cel-D7) and ^{13}C NMR (Fig. 12 for cel-D2; Fig.13 for cel-D7), and high resolution mass spectrometry
30 (Fig. 14 for cel-D2; Fig. 15 for cel-D7).

To confirm that these derivatives of celastrol retain the ability to disrupt HSP90/CDC37 interaction, we first did Western blot analysis of HSP90 after immunoprecipitation of CDC37 from HepG2 cell lysates, which demonstrated that immunoprecipitation of CDC37 pulled down HSP90 as expected. Treatment of HepG2 cells for 6 hours with celastrol or its three derivatives

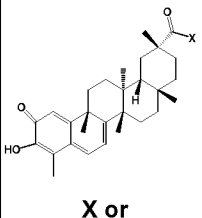
at 5 μ M each decreased the amount of HSP90 in the immunoprecipitated CDC37 complex. Our results showed that HSP90 and CDC37 formed a complex in vitro and that all four chemicals disrupted their direct interaction.

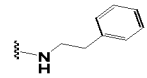
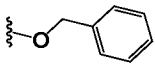
5 Celastrol and its derivatives preferentially inhibited viability of HCC cells compared to normal hepatocytes

To test our hypothesis that HSP90/CDC37 antagonists are feasible anti-tumor agents in the treatment of HCC, their selective cytotoxicity against HCC cells (HepG2, Huh7, and Hep3B) were tested compared to normal hepatocytes (Hu8114, Hu4175, and Hu8130, obtained from
10 three donors with non-diseased liver). Western blot analysis confirmed that only HCC cells express high levels of CDC37, whereas normal hepatocytes express undetectable levels of CDC37 (Fig. 2A). Accordingly, treatment of these HCC cell lines and normal hepatocytes with celastrol or its derivatives for 3 days showed greater inhibition of cell viability in HCC cell lines compared to normal hepatocytes, with several fold lower IC_{50} s in HCC cell lines than in normal
15 hepatocytes for each compound (Table 3; Fig. 8). Light microscopic examination demonstrated greater toxicity towards HCC cells compared to normal hepatocytes (representative images for treatment with cel-D7 are shown in Fig. 2B). Each of these compounds also induced apoptosis in HCC cell lines (representative images for Huh7 cells are shown in Fig. 2C). Our data indicate that modification of the carboxylic acid group of celastrol retained HSP90/CDC37 antagonist
20 activity, as well as anti-tumor activity in HCC cell lines. Notably, celastrol and pristimerin, both with non-aromatic substituents, show greatest activity against HCC cells, and are also considerably more toxic against normal hepatocytes. Cel-D2 and cel-D7, with aromatic phenyl substituents, exhibited reduced activity against HCC cells; with cel-D7 being the least toxic against normal hepatocytes.

25

Table 3. Structure and activity of celastrol and its derivatives

Compound	 X or	IC_{50} in HCC cell lines (μ M)			IC_{50} in normal hepatocytes (μ M)
		HepG2	Huh7	Hep3B	
celastrol		1.22 $\pm$ 0.12	1.07 $\pm$ 0.13	0.30 $\pm$ 0.08	2.06-3.08

pristimerin		1.7±0.21	0.68±0.05	0.85±0.08	3.87-5.33
cel-D2		3.58±0.32	1.04±0.05	1.06±0.10	5.9-16.8
cel-D7		4.26±0.23	2.15±0.14	2.77±0.32	15.66-23.95

Celastrol and its derivatives induced degradation and inhibited phosphorylation of HSP90/CDC37 client protein kinases in HCC cell lines

5 To study the molecular events resulting from treatment with celastrol and its derivatives, we detected the levels and phosphorylation status of several HSP90/CDC37 client protein kinases that are known to be highly activated in HCC, including the Raf family proteins, AKT, MEK1/2, CDK4, and EGFR. Treatment of HepG2, Huh7, and Hep3B cells with celastrol and its derivatives for 6 hours reduced the protein levels and phosphorylation levels of all the

10 HSP90/CDC37 client protein kinases studied, compared to DMSO control (Fig. 3). These effects were dose-dependent, with 10 μ M concentration of each compound causing greater reductions in expression and phosphorylation levels for almost all protein kinases compared to treatment with 1 μ M of each compound. EGFR was not detected in HepG2 cells, but showed decreased levels after treatment with all compounds in Huh7 cells (with dose-dependence seen only with

15 pristimerin and cel-D7). CDC37 levels did not change after treatment with any compound.

Celastrol and its derivatives inhibited growth of orthotopic HCC patient-derived xenografts

20 The anti-tumor effects of celastrol and its derivatives were next evaluated in orthotopic HCC patient-derived xenografts. Tumors from three HCC patients (HCC-1, HCC-2, and HCC-3) were confirmed to express high levels of CDC37 compared to their matched non-tumor liver tissues (Fig. 4A). Preliminary limited toxicity studies in NSG mice suggested the maximum tolerate dose was 4 mg/Kg for celastrol; 1 mg/Kg for pristimerin; 8 mg/Kg for cel-D2; and 8 mg/Kg for cel-D7. At these respective doses, none of the four compounds resulted in significant

25 toxicity or any noticeable discomfort to the mice, suggesting that these doses were well tolerated. These doses were therefore used for treatment, which was initiated within one week after orthotopic transplant of the xenografts stably expressing the luciferase reporter gene.

Based on weekly monitoring of luciferase signal in vivo, all four compounds significantly

inhibited growth of all three orthotopic HCC xenografts (compared to saline treated controls) at the end of the 3-weeks treatment period (Fig. 4B-D; Fig. 9). Measurement of final tumor volumes at the end of the treatment period consistently showed that all compounds caused significant reductions in tumor volumes in all three orthotopic HCC xenografts (when each treatment group is separately compared to saline control group) (Fig. 4E; $P < 0.05$). On average across all three HCC xenografts, celastrol reduced tumor volumes by 2-5 fold; pristimerin by 5-7 fold; cel-D2 by 1.5-3.5 fold; and cel-D7 by 1.8-3.2 fold. The in vivo data were consistent with the in vitro data, with celastrol and pristimerin showing greater anti-tumor activity than cel-D2 and cel-D7. Of note, tumor mass was absent from one of five mice in the group with HCC-1 xenografts after celastrol treatment, and from one of five mice in all three groups with HCC-1, HCC-2, or HCC-3 xenografts after pristimerin treatment.

In vivo toxicology analysis revealed that celastrol and pristimerin caused slight decreases in the body weight of treated mice, whereas cel-D2 and cel-D7 did not affect body weight of treated mice (compared to saline treated controls) (Fig. 4F and **Table 4**). In addition, white blood cells were elevated by about 2.5-folds in the celastrol treatment group (16.32 ± 3.23 K/ μ L) as compared to the saline control group (6.34 ± 2.34 K/ μ L). Correspondingly, mice treated with celastrol have enlarged spleens (**Table 4**). We also noted that pristimerin treatment significantly elevated the blood AST and ALT level as compared to saline control group (**Table 5**), suggesting that pristimerin may negatively impact liver functions. However, cel-D2 and cel-D7 treatments did not result in any significant changes in body weight or blood chemistries. These in vivo observations were consistent with our in vitro data that celastrol and pristimerin are more toxic to normal hepatocytes.

Table 4. Selected organ weights after treatment in vivo

Total body weights and selected organ weights of BALB/cJ mice (n=4/group) treated with different compounds. Data represent mean $\pm$ SD. (* $p < 0.05\%$, compared to saline group.)

Organ	saline	celastrol	pristimerin	cel-D2	cel-D7
Brain	0.41 $\pm$ 0.03	0.4 $\pm$ 0.01	0.39 $\pm$ 0.02	0.42 $\pm$ 0.01	0.41 $\pm$ 0.01
Heart	0.12 $\pm$ 0.01	0.13 $\pm$ 0.01	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.13 $\pm$ 0.01
Kidney	0.39 $\pm$ 0.03	0.37 $\pm$ 0.02	0.36 $\pm$ 0.04	0.4 $\pm$ 0.02	0.39 $\pm$ 0.03
Liver	1.1 $\pm$ 0.06	1.14 $\pm$ 0.07	0.98 $\pm$ 0.09	1.13 $\pm$ 0.09	1.05 $\pm$ 0.13

Lung	0.17±0.02	0.17±0.01	0.15±0.01	0.18±0.01	0.17±0.02
Spleen	0.11±0.01	0.19±0.03*	0.11±0.01	0.12±0.01	0.12±0.02
Body weight	24.17±0.73	21.42±0.81*	20.35±0.82*	23.85±1.43	22.83±0.97

Table 5. Selected toxicity results after treatment in vivo

Representative toxicological data of BALB/cJ mice (n=4/group) treated with different compounds. WBC: white blood cells; RBC: red blood cells; HGB: hemoglobin; ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen. Data represent mean ± SD. (* p < 0.05%, compared to saline group.)

5

Selected Parameters	saline	celastrol	pristimerin	cel-D2	cel-D7	Normal Range
WBC (K/ μ L)	6.34±2.34	16.32±3.23*	4.26±1.49	4.04±2.09	4.49±2.21	3.2-12.8
RBC (M/ μ L)	9.58±0.55	9.17±0.50	8.23±0.89	6.96±2.44	7.23±2.41	8.71-11.6
HGB (g/dL)	14.83±0.49	13.96±0.39	12.70±1.36	11.10±3.03	11.98±2.35	13-17.2
Albumin (g/dL)	2.78±0.24	2.78±0.05	2.93±0.28	2.56±0.15	2.55±0.13	2.5-2.9
Creatine (mg/dL)	0.35 ±0.10	0.45±0.06	0.15±0.06	0.28±0.09	0.38±0.10	0.25-0.47
ALP(IU/L)	89.25±29.92	48.75±4.50	103.8±26.52	78.00±19.70	92.50±9.68	82-172
AST(U/L)	201.8±60.07	169.0±94.63	804.8±753.8*	207.75±62.45	171.3±84.0	57-382
ALT(U/L)	145.0±63.69	85.75±68.09	475.8±266.4*	193.8±115.7	92.00±94.08	21-187
Total	0.05 ±0.10	0.15±0.10	0.00±0.00	0.00±0.00	0.15±0.24	0.03-0.23

Bilirubin (mg/dL)						
Total protein (g/dL)	5.55±0.68	5.70±0.00	5.35±0.34	5.13±0.17	4.85±0.17	4.4-5.9
BUN (mg/dL)	22.00±5.60	19.50±1.73	18.75±2.50	18.50±1.29	18.00±0.82	17-30

Celastrol and its derivatives induced apoptosis and degradation of HSP90/CDC37 associated client protein kinases in orthotopic HCC patient-derived xenografts

5 Similar to our in vitro observations with HCC cell lines, all four compounds induced cell apoptosis in all the orthotopic HCC xenografts when harvested tumors were analyzed by TUNEL staining (representative images for HCC-3 xenografts are shown in Fig. 5). Celastrol and pristimerin showed more extensive staining of apoptotic cells than cel-D2 and cel-D7, consistent with the greater anti-tumor activities observed with the former two compounds.

10 Western blot analysis of HSP90/CDC37 client protein kinases demonstrated that all four compounds induced degradation and inhibited phosphorylation of most of the studied kinases (representative images for HCC-3 are shown in Fig. 6). Pristimerin in particular showed strongest effects on AKT, MEK1/2 and EGFR, whereas celastrol showed strongest effect on B-Raf. CDC37 expression in these xenografts were maintained (compared to original HCC tumor

15 levels), and expression levels were unaffected by treatment with all four compounds.

Discussion

HSP90 and its co-chaperone CDC37 are key factors in the chaperone-kinome pathway that plays permissive roles in the oncogenesis of multiple types of tumors. We hypothesized that

20 the direct disruption of HSP90 interaction with CDC37 by the small molecule celastrol would achieve desirable anti-tumor effects. Indeed, celastrol and three of its derivatives were successfully shown to disrupt HSP90 and CDC37 interaction in HCC cells; to inhibit the growth of secondary HCC cell lines in vitro; and to inhibit the growth of orthotopic HCC patient-derived xenografts in vivo.

25 The observed anti-tumor activities of celastrol and its derivatives both in vitro and in vivo promoted degradation and decreased phosphorylation of protein kinases that are highly

activated in HCC cells, such as Raf family proteins, AKT, MEK1/2, CDK4, and EGFR. This ability to simultaneously disrupt multiple oncogenic signaling pathways suggests that these HSP90/CDC37 antagonists are potentially broad spectrum inhibitors that would be beneficial for treating the heterogeneous subtypes of HCC. Our results suggest that HSP90/CDC37 antagonists can effectively inhibit the above (and other) pathways simultaneously, offering the benefit of treating a larger percentage of HCC patients without the need to administer chemotherapeutic cocktails that would also increase side effects.

An additional benefit resulting from simultaneous inhibition of a wide array of oncogenic kinase pathway is the potential to help overcome drug resistance that is often associated with the activation of one or more pathways. For example, acquired drug resistance to sorafenib (targeting angiogenesis and Raf/MEK/ERK signaling pathways) has been attributed to the activation of PI3K/AKT and EGFR; adaptive drug resistance to EGFR-targeted therapies have been associated with activation of PI3K/AKT pathway; whereas therapies targeting AKT/mTOR leads to Raf/MEK/ERK pathway activation through a PI3K-dependent feedback loop. Thus, the use of HSP90/CDC37 antagonists either by themselves or in combination therapy with other pathway-specific inhibitors may potentially circumvent the development of acquired drug resistance by inhibiting multiple pathways simultaneously, and at the same time increase drug sensitivity. This is particularly beneficial for HCC, which is highly resistant to currently used chemotherapeutic drugs, making successful treatment a clinical challenge.

In our attempt to develop new and more efficacious derivatives of celastrol, we synthesized a total of seven derivatives (cel-D1 to cel-D7, with cel-D5 being pristimerin) by introducing different substituents (aromatic or non-aromatic) via amide or ester bond to the chemically active carboxylic acid group of celastrol. The structures and IC₅₀s of all seven derivatives are shown in Fig. 7. Celastrol, pristimerin, cel-D2 and cel-D7 were selected for further studies based on their greater preferential activities against HCC cells compared to normal hepatocytes. Among them, the data suggest that celastrol and pristimerin have greater anti-tumor efficacy but also greater toxicity both in vitro and in vivo (observed as lower IC₅₀ to normal hepatocytes in vitro and greater weight loss in vivo); whereas cel-D2 and cel-D7 have slightly reduced anti-tumor efficacy, but also have reduced toxicity (observed as higher IC₅₀ to normal hepatocytes in vitro and absence of weight loss in vivo). Celastrol treatment also caused enlarged spleen and elevated white blood cells; whereas pristimerin treatment caused significant liver impairment (elevated AST and ALT level). At the molecular level, celastrol and pristimerin showed greatest inhibitory effects on the client protein kinases, which may underlie their greater apoptotic effects to both malignant and normal hepatocytes. The structure-activity-

relationship (SAR) study suggests that modifications of the carboxylic acid group of celastrol do not drastically affect its ability to disrupt HSP90/CDC37 inhibition; however, derivatives with aromatic phenyl substituents (cel-D2 and cel-D7) appear to induce less marked apoptosis and may therefore be less toxic to normal cells compared to derivatives with non-aromatic alkyl groups. The clinical translation of celastrol and pristimerin may be limited due to their toxicity.

The use of orthotopic patient-derived xenografts in our study provides a high predictive value of the response of HCC patients to celastrol and its derivatives. The human origin of these xenografts more accurately reflect the response rates; in fact, responses to chemotherapy in patient-derived xenografts have been reported to resemble the response rates of monotherapy in clinical trials. Orthotopic models have also been shown to be predictive of a patient's response to chemotherapy. Our xenografts are established from HCC patients with over-expression of CDC37, which we confirmed to be maintained in the xenografts.

In summary, we demonstrated that HSP90/CDC37 antagonists can be used for the treatment of HCC, which are typically resistant to standard chemotherapeutic agents. They are broad spectrum agents, being able to simultaneously disrupt multiple oncogenic pathways that are critical in development of the heterogeneous subtypes of HCC. Thus, HSP90/CDC37 antagonists will be effective as monotherapy or as combination therapy with other conventional agents.

CLAIMS

That which is claimed is:

1. A compound selected from:

Compound 2: (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-N-phenethyl-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 3: (2R,4aS,6aS,12bR,14aS,14bR)-N,N-diethyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide; and

Compound 4: (2R,4aS,6aS,12bR,14aS,14bR)-N-tert-butyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide.

2. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier.

3. A method of antagonizing the interaction of HSP90 with CDC37 in a biological sample or a patient, the method comprising:

contacting the biological sample or administering to the patient a compound selected from:

Compound 1: (2R,4aS,6aS,12bR,14aS,14bR)-N-benzyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 2: (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-N-phenethyl-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 3: (2R,4aS,6aS,12bR,14aS,14bR)-N,N-diethyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide; and

Compound 4: (2R,4aS,6aS,12bR,14aS,14bR)-N-tert-butyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide.

4. The method of Claim 3, wherein the Compound is selected from: Compound 1 and Compound 2.
5. The method of Claim 4, wherein the Compound is Compound 2.
6. The method of Claim 3, wherein antagonizing the interaction of HSP90 with CDC37 results in treatment of a disease or disorder that is mediated or sustained through the interaction of HSP90 with CDC37.
7. The method of Claim 3, wherein the disease or disorder is a cancer.
8. The method of Claim 7, wherein the cancer is hepatocellular carcinoma.
9. A method of antagonizing the interaction of HSP90 with CDC37 in an individual having cancer, the method comprising:
administering to the individual, at a dose effective for antagonizing the interaction of HSP90 with CDC37 in cancer cells, a compound selected from:
Compound 1: (2R,4aS,6aS,12bR,14aS,14bR)-N-benzyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydricene-2-carboxamide;
Compound 2: (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-N-phenethyl-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydricene-2-carboxamide;
Compound 3: (2R,4aS,6aS,12bR,14aS,14bR)-N,N-diethyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydricene-2-carboxamide; and
Compound 4: (2R,4aS,6aS,12bR,14aS,14bR)-N-tert-butyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydricene-2-carboxamide.
10. The method of Claim 9, wherein the Compound is selected from: Compound 1 and Compound 2.
11. The method of Claim 10, wherein the Compound is Compound 2.

12. The method of claim 9, wherein the cancer is a carcinoma.
13. The method of claim 12, wherein the carcinoma is hepatocellular carcinoma (HCC).
14. A method of treating an individual having cancer, the method comprising:
administering to the individual, at a dose effective for reducing the number of cancer cells in the individual, a compound selected from:
Compound 1: (2R,4aS,6aS,12bR,14aS,14bR)-N-benzyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;
Compound 2: (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-N-phenethyl-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;
Compound 3: (2R,4aS,6aS,12bR,14aS,14bR)-N,N-diethyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide; and
Compound 4: (2R,4aS,6aS,12bR,14aS,14bR)-N-tert-butyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide.
15. The method of Claim 14, wherein the Compound is selected from: Compound 1 and Compound 2.
16. The method of Claim 15, wherein the Compound is Compound 2.
17. The method of claim 14, wherein the cancer is a carcinoma.
18. The method of claim 17, wherein the carcinoma is hepatocellular carcinoma (HCC).
19. A method of inhibiting the interaction of HSP90 with CDC37 in an individual having hepatocellular carcinoma (HCC), the method comprising:
administering to the individual, in an amount effective for inhibiting the interaction of HSP90 with CDC37 in HCC cancer cells, a compound selected from:

Compound 1: (2R,4aS,6aS,12bR,14aS,14bR)-N-benzyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 2: (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-N-phenethyl-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 3: (2R,4aS,6aS,12bR,14aS,14bR)-N,N-diethyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 4: (2R,4aS,6aS,12bR,14aS,14bR)-N-tert-butyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide.

Compound 5: methyl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylate;

Compound 6: propan-2-yl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylate; and

Compound 7: benzyl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylate.

20. A method of treating an individual having hepatocellular carcinoma (HCC), the method comprising:

administering to the individual, at a dose effective for reducing the number of HCC cancer cells in the individual, a compound selected from:

Compound 1: (2R,4aS,6aS,12bR,14aS,14bR)-N-benzyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 2: (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-N-phenethyl-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 3: (2R,4aS,6aS,12bR,14aS,14bR)-N,N-diethyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide;

Compound 4: (2R,4aS,6aS,12bR,14aS,14bR)-N-tert-butyl-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydronicene-2-carboxamide.

Compound 5: methyl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylate;

Compound 6: propan-2-yl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylate; and

Compound 7: benzyl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylate.

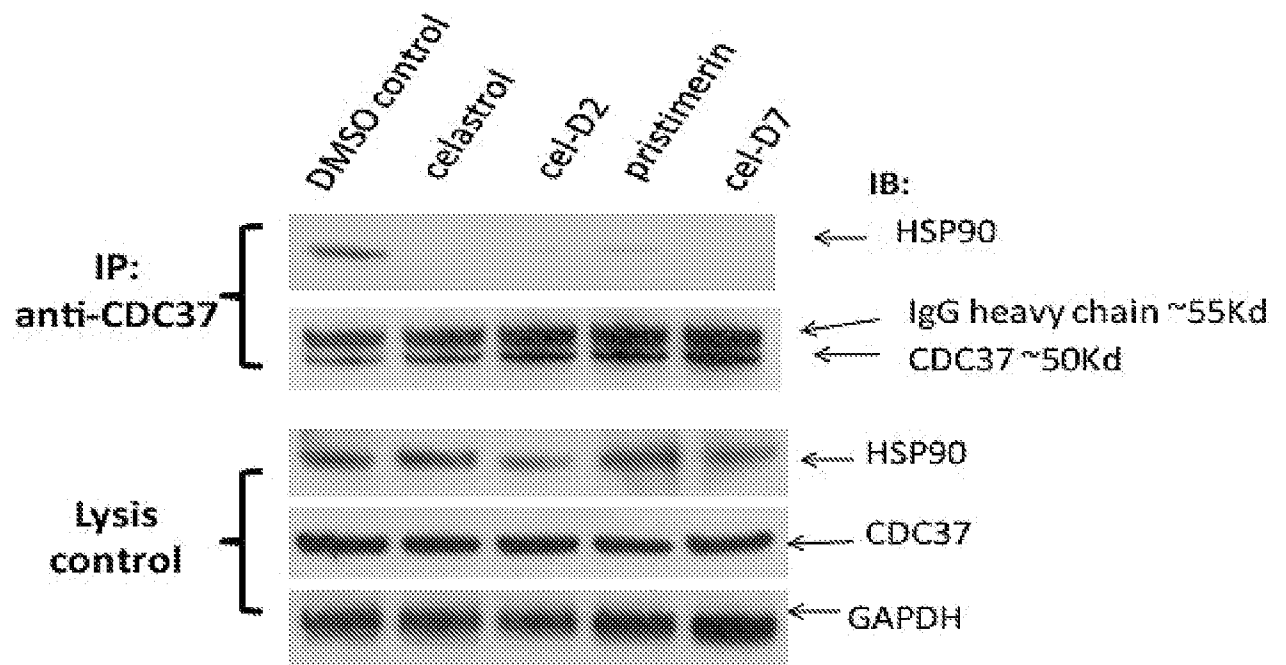
21. A method of making Compound 2, Compound 3, or Compound 4, the method comprising:

contacting (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydronicene-2-carboxylic acid (Celastrol) with phenethylamine (2-phenylethylamine), diethylamine (ethanamine), or tert-butylamine (2-propanamine).

22. The method of Claim 21, wherein the reaction is run in a polar aprotic solvent.

23. The method of Claim 21, wherein the reaction is run in the presence of a coupling reagent and a base.

Figure 1



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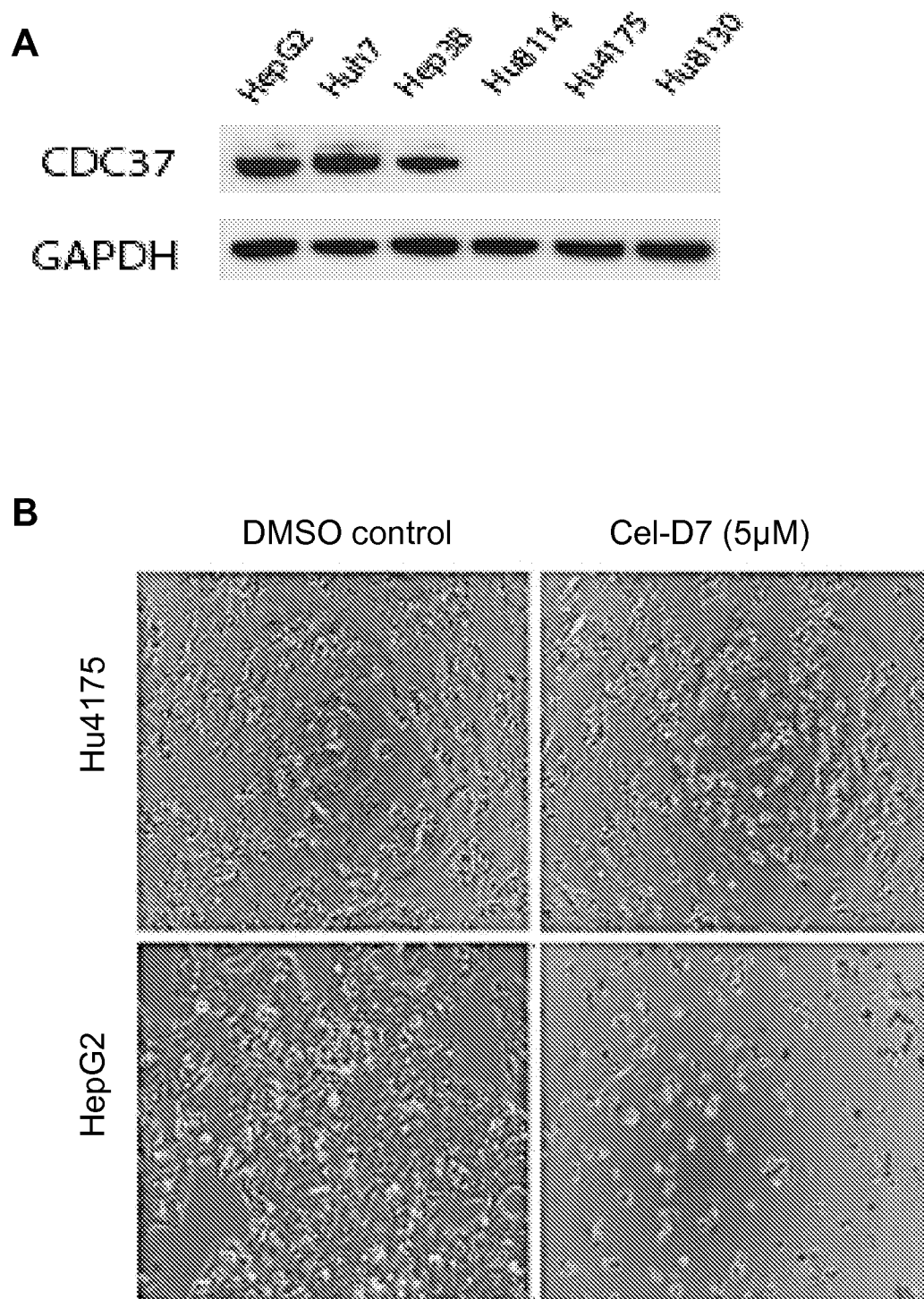
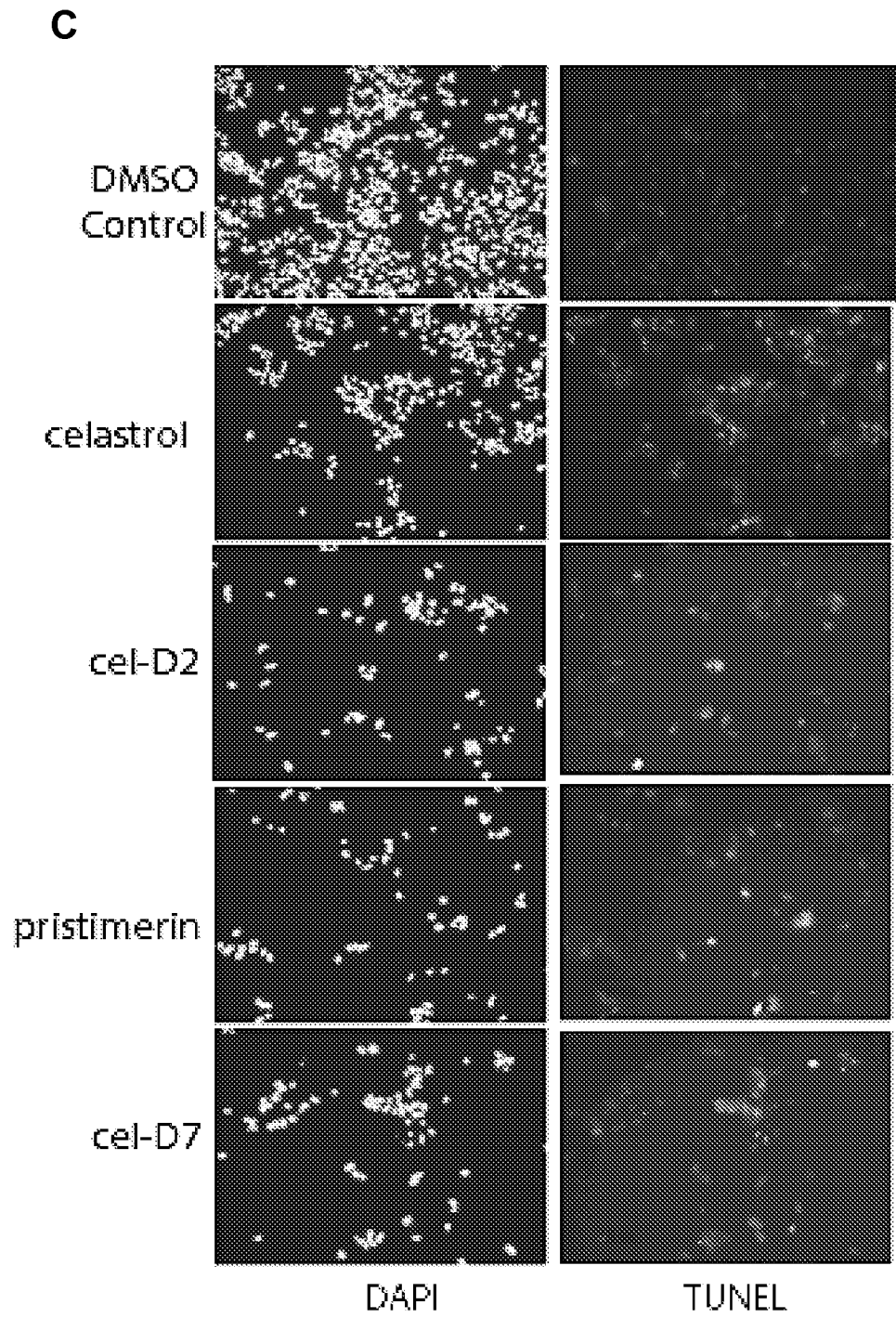
Figure 2

Figure 2



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Figure 3

A

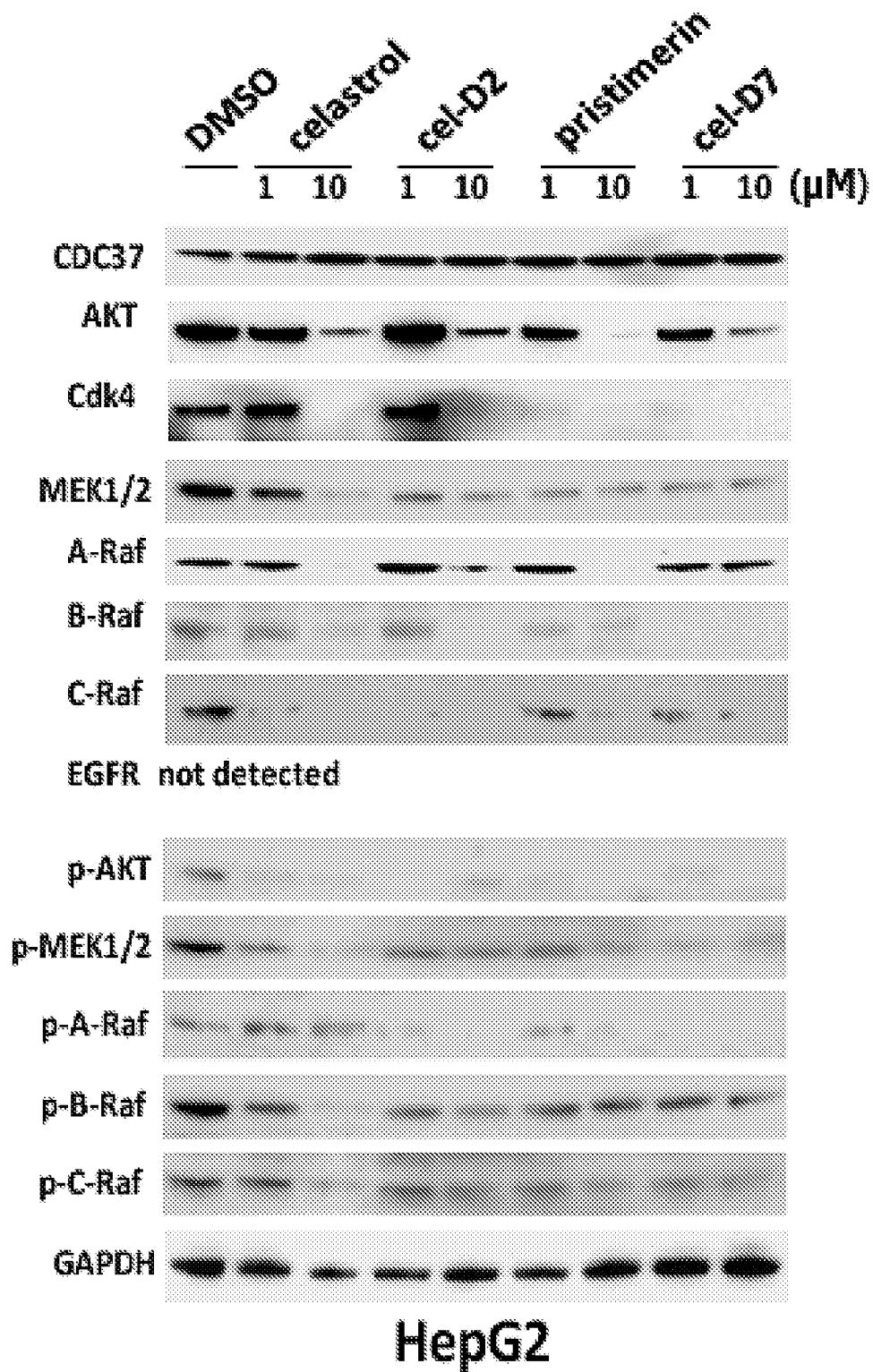
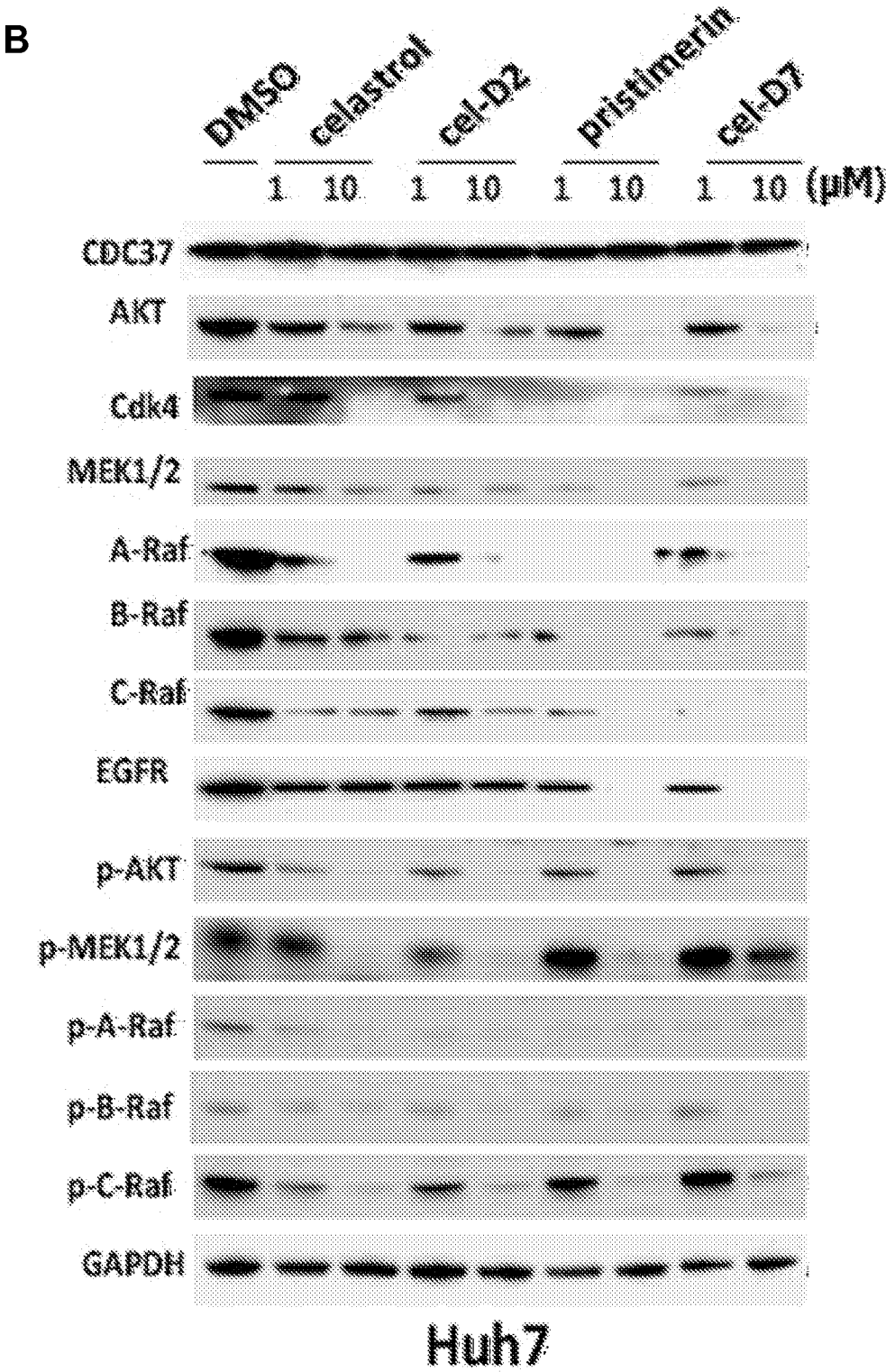
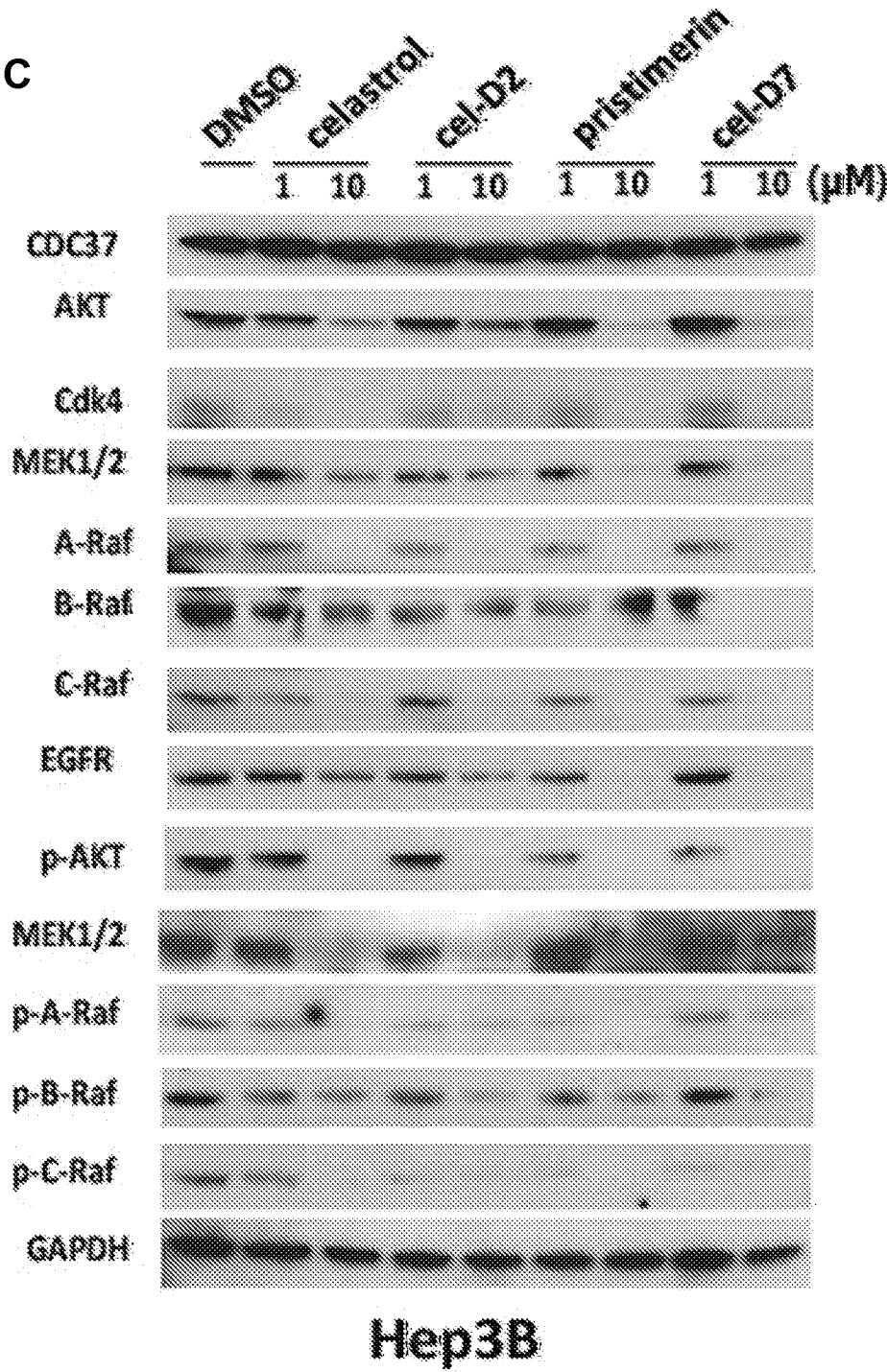


Figure 3



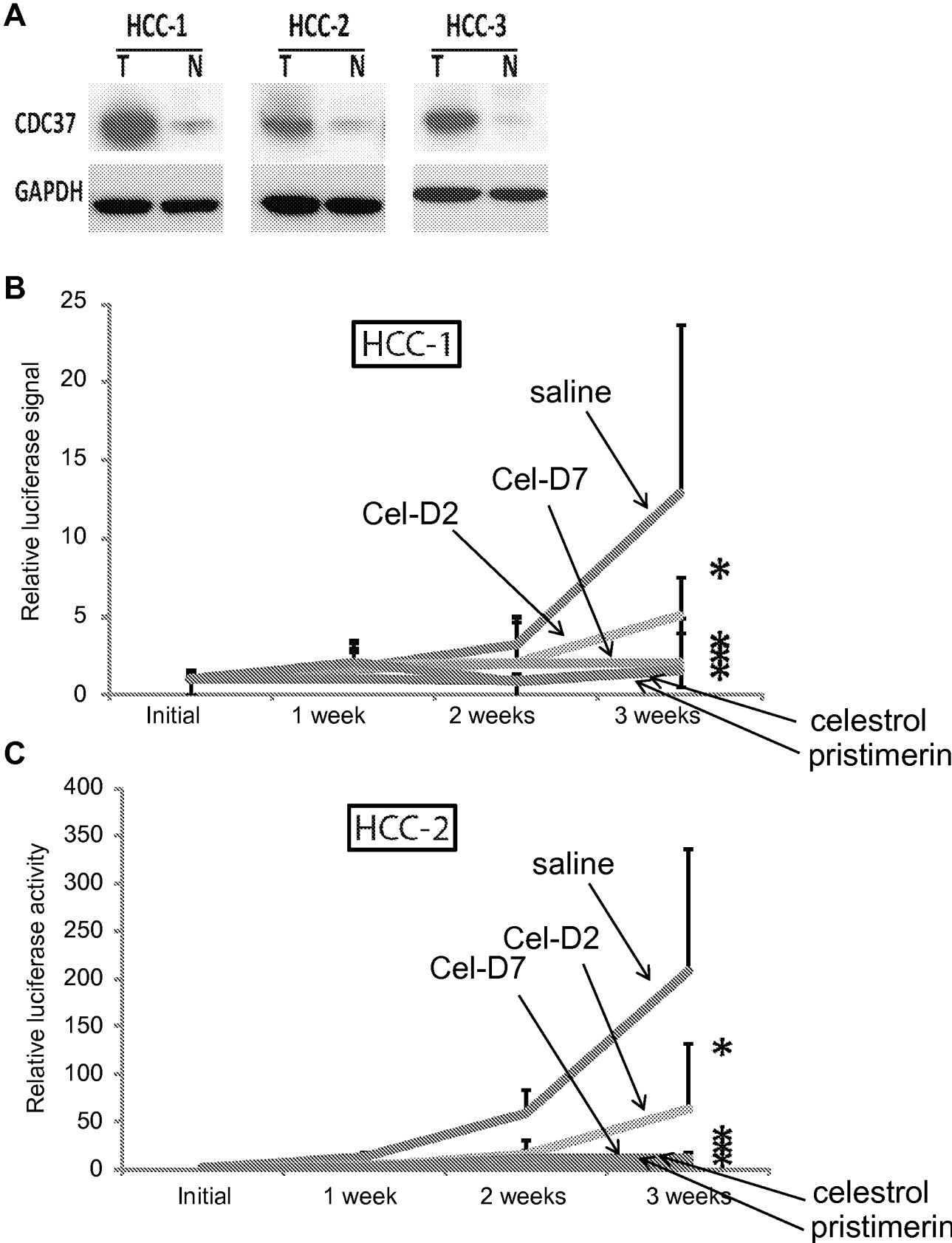
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Figure 3



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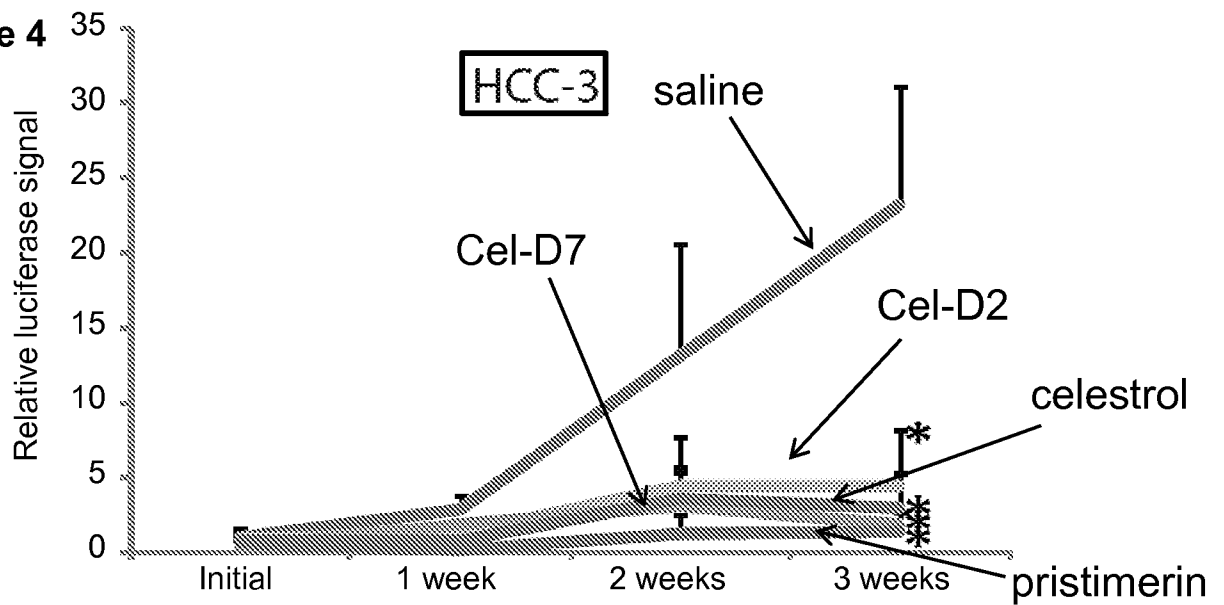
Figure 4



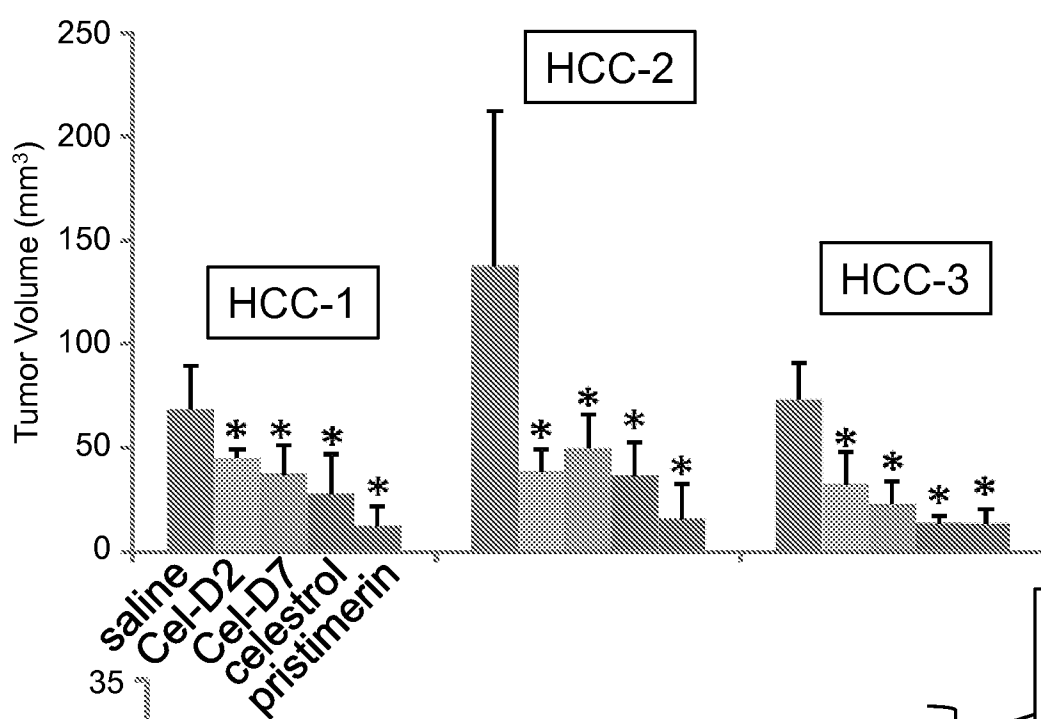
8/26

Figure 4

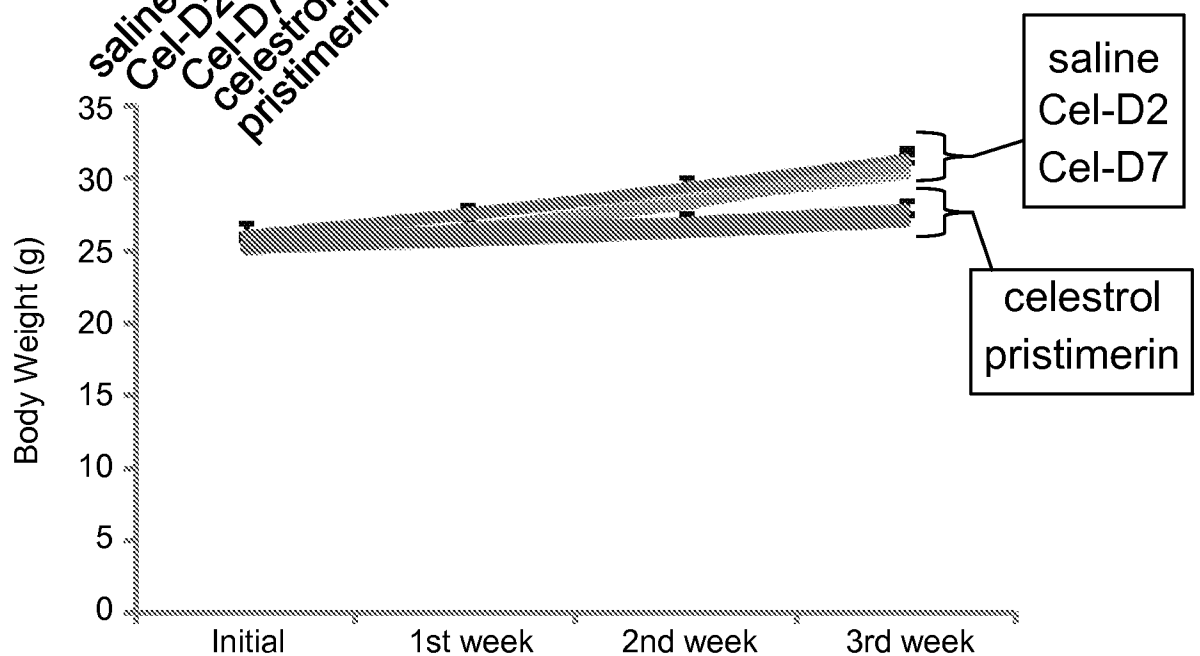
D



E

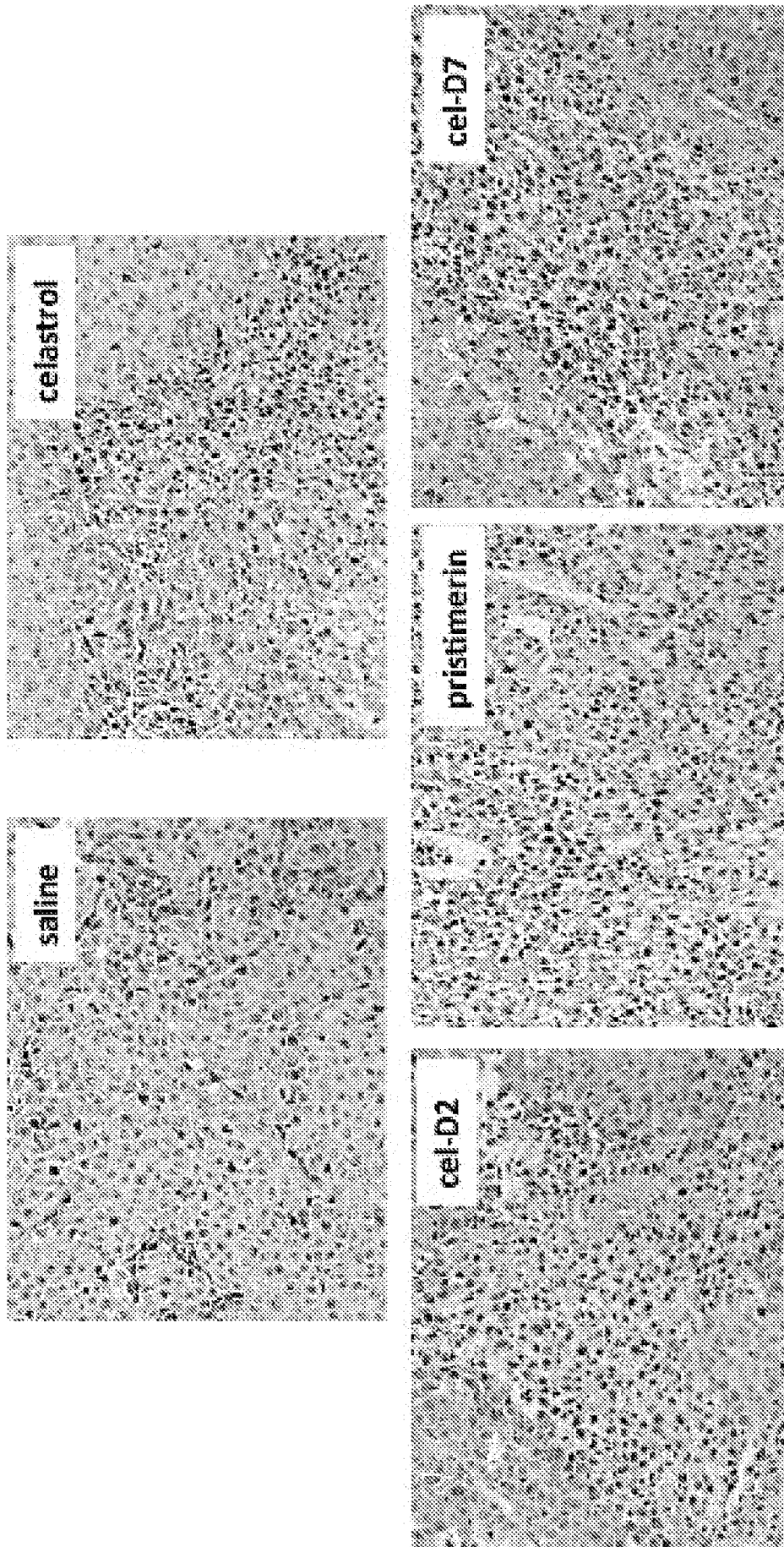


F



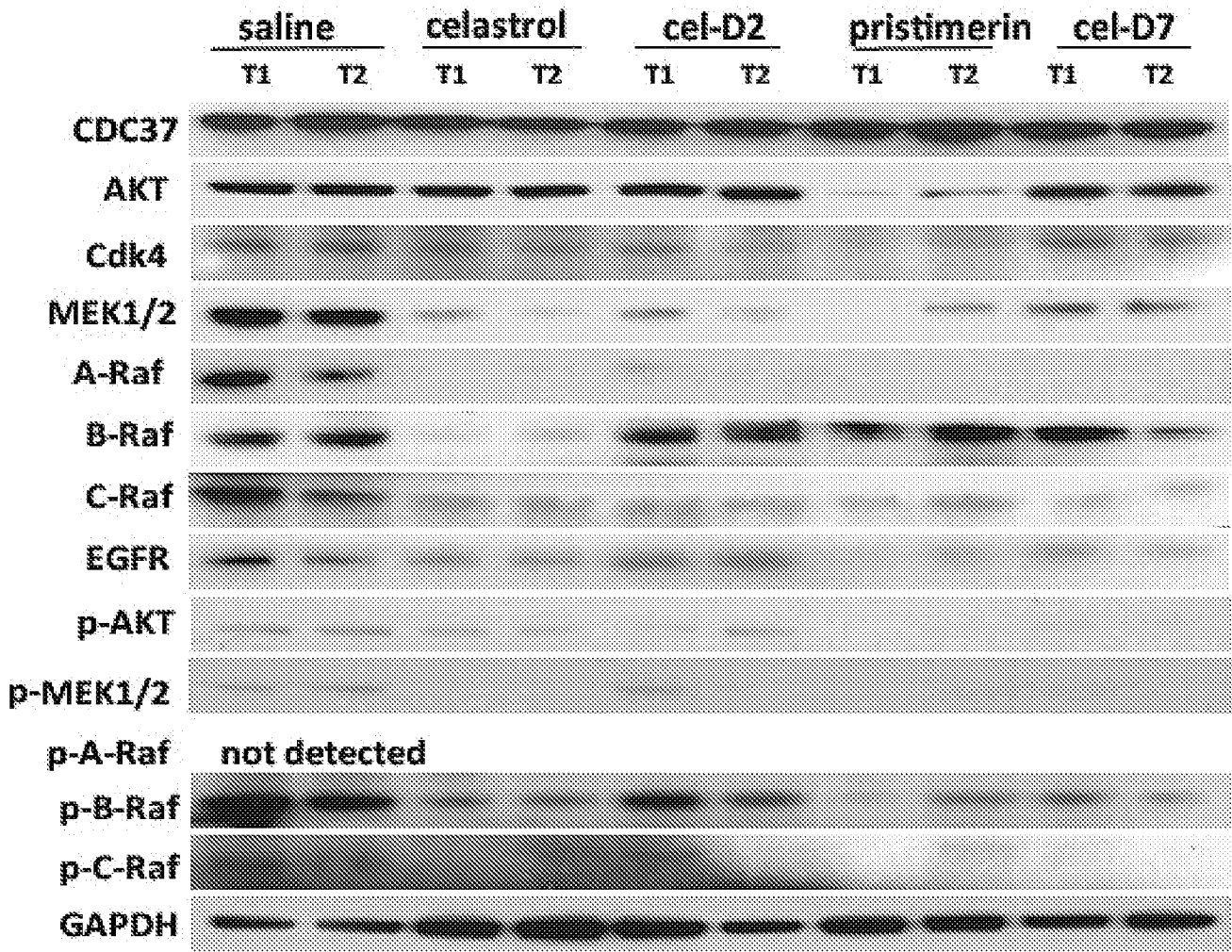
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Figure 5



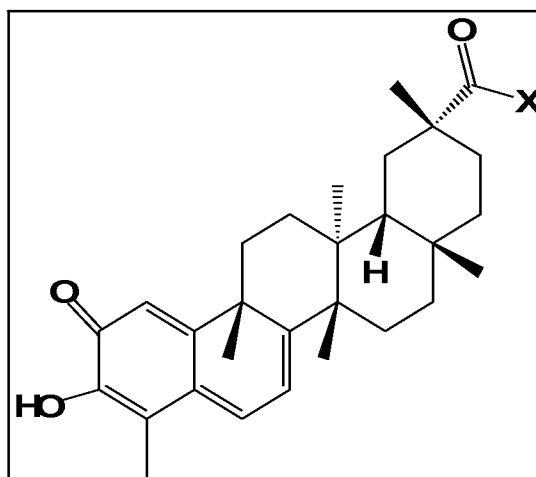
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Figure 6



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Figure 7



Compound		IC ₅₀ to HCC cell lines (μM)	IC ₅₀ to normal hepatocytes (μM)
<u>celastrol</u>		0.3-1.22	2.06-3.08
cel-D1		0.59-1.7	0.88-2.45
cel-D2		1.04-3.58	5.9-16.8
cel-D3		3.59-4.89	8.91-9.35
cel-D4		3.13-4.67	4.19-7.1
cel-D5		0.68-1.7	3.87-5.33
cel-D6		0.44-1.19	2.03-3.19
cel-D7		2.15-4.26	15.66-23.95

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Figure 8

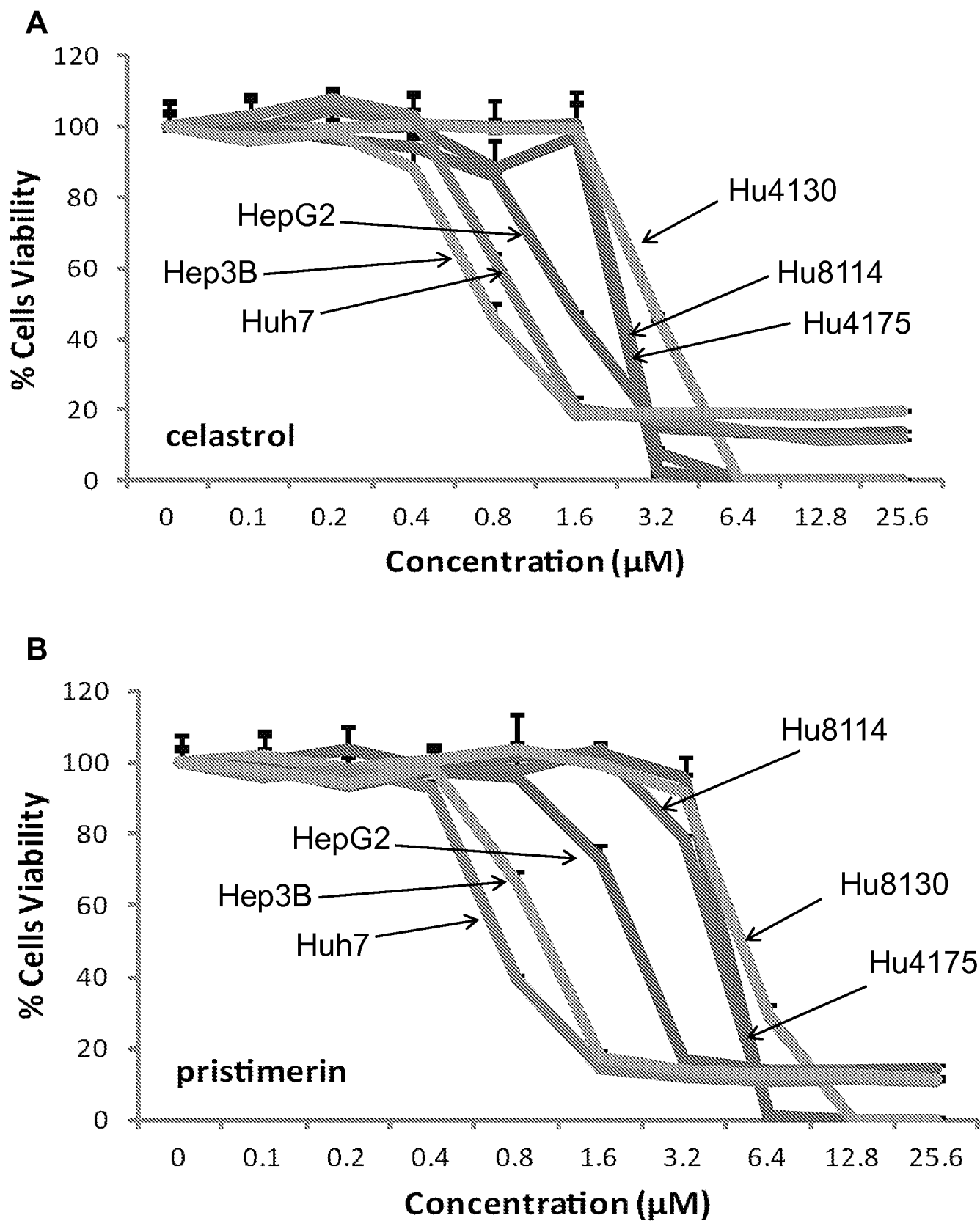
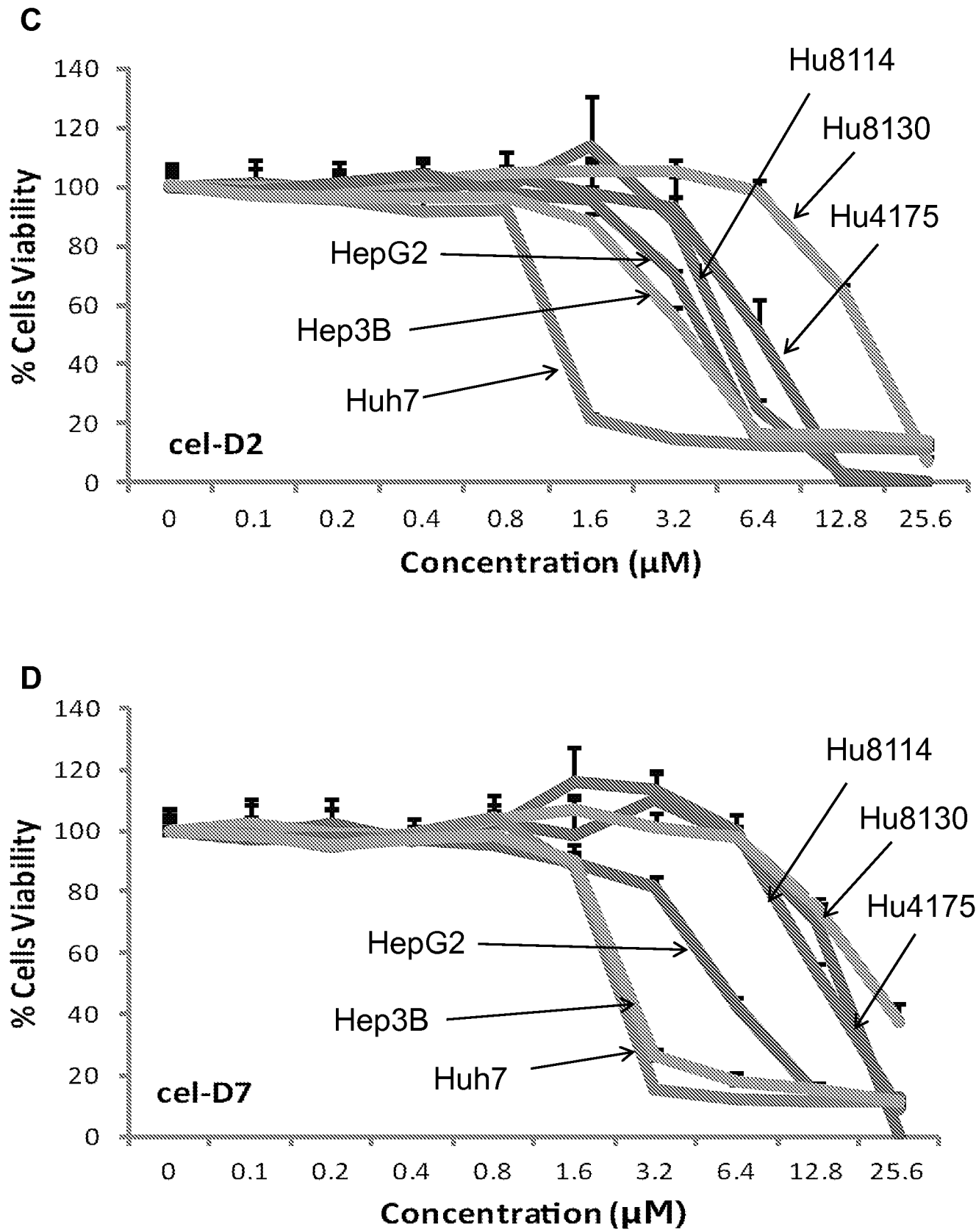


Figure 8



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Figure 9

A

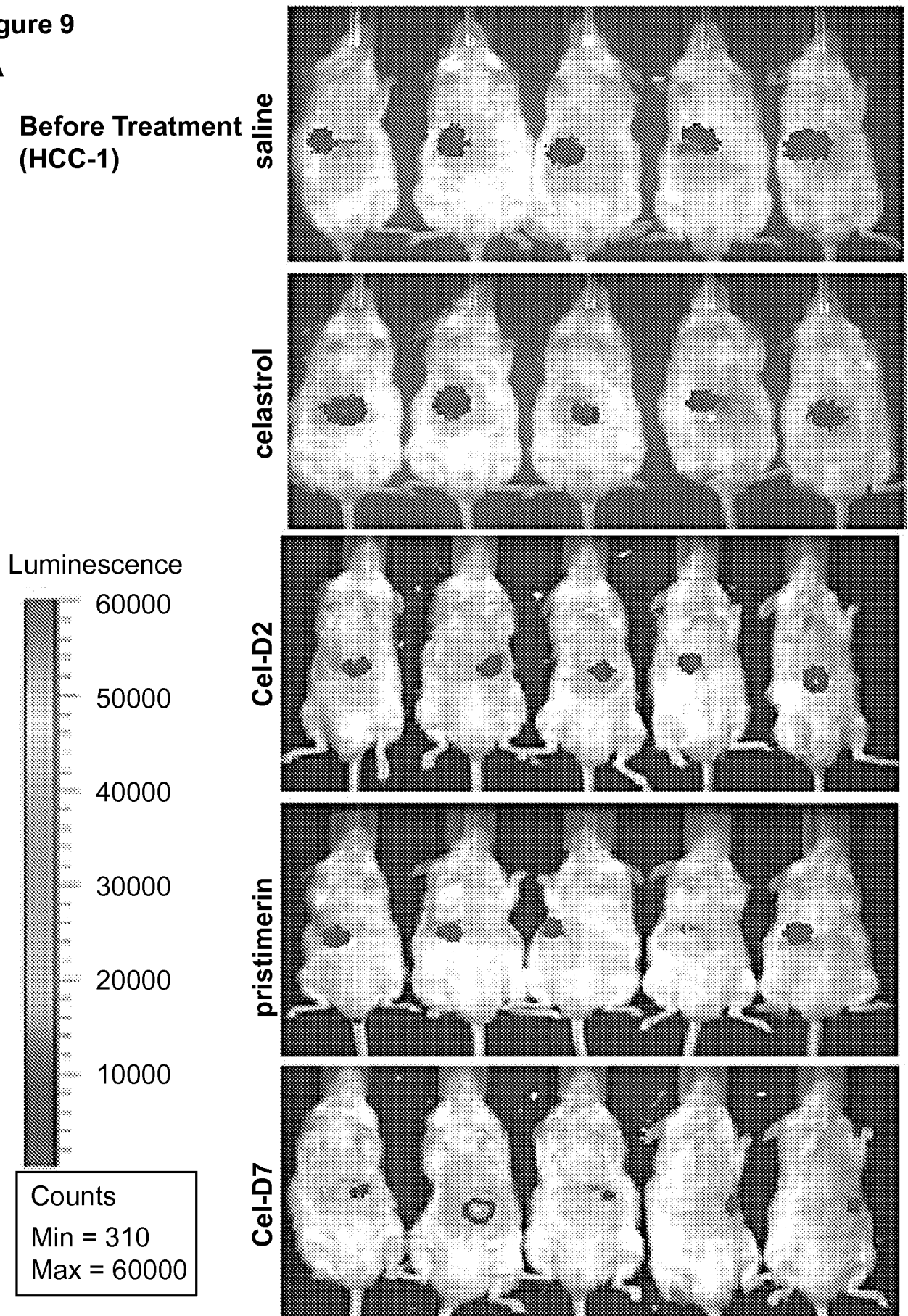
Before Treatment
(HCC-1)

Figure 9

B

After Treatment
(HCC-1)

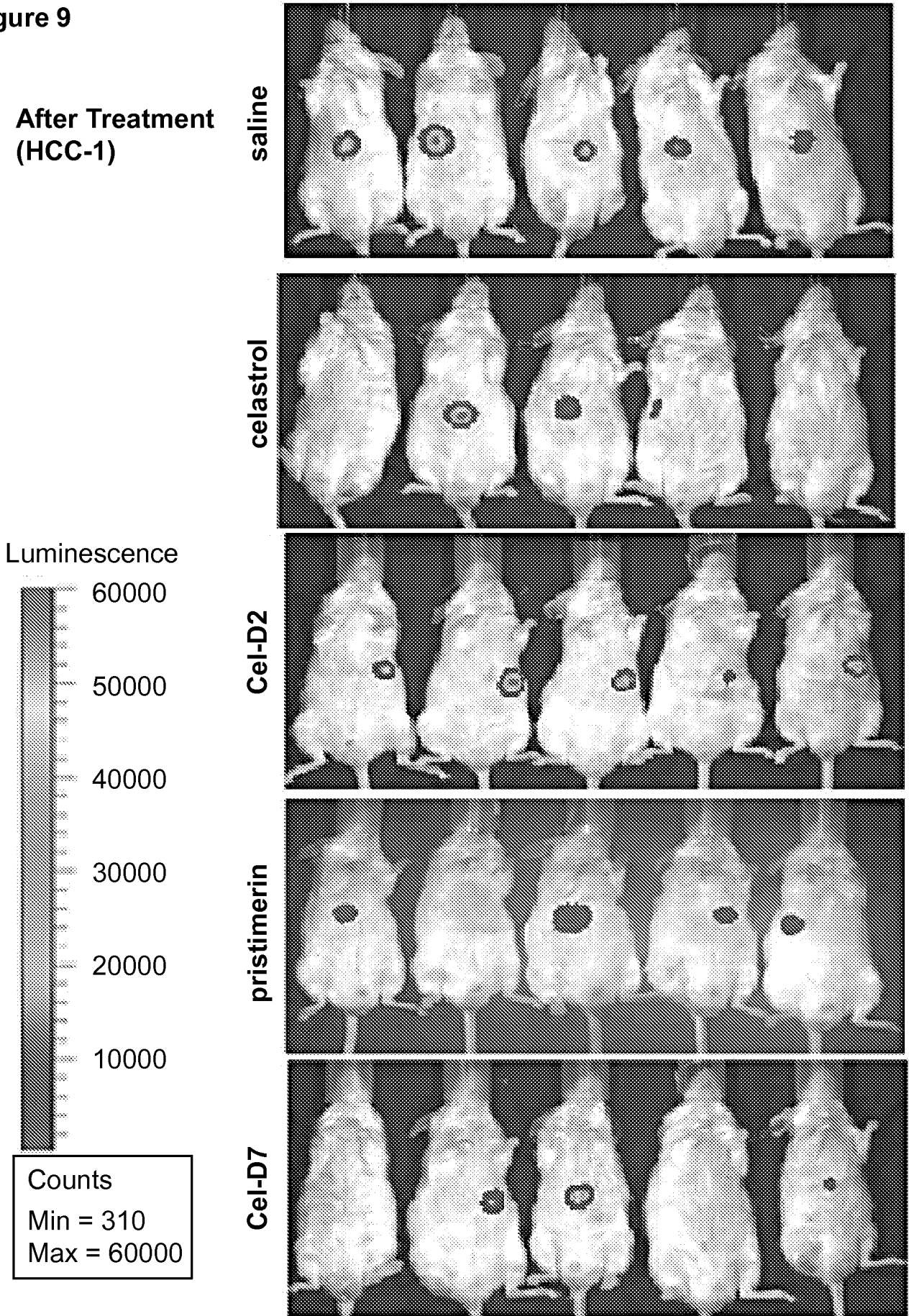


Figure 9

C

Before Treatment
(HCC-2)

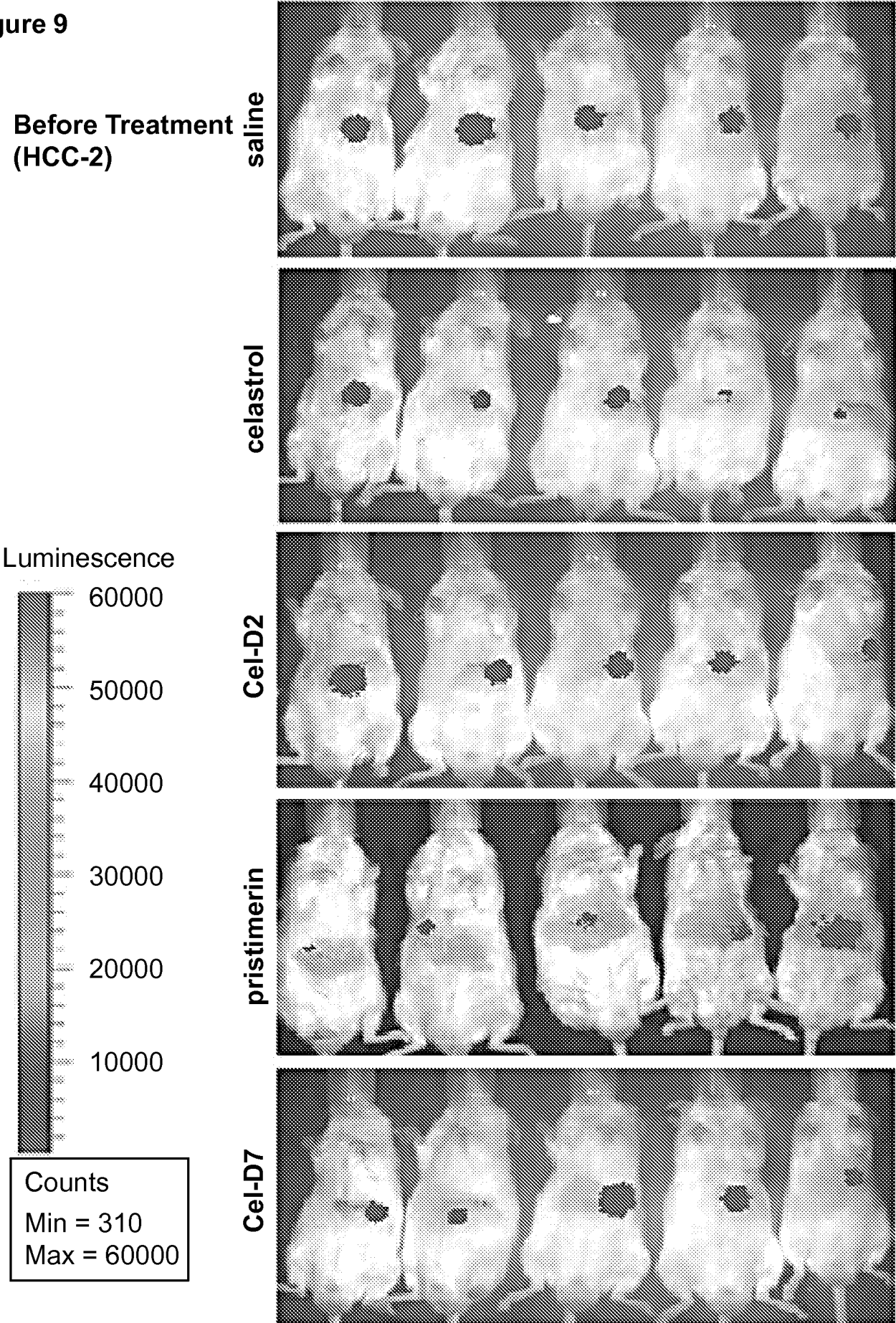
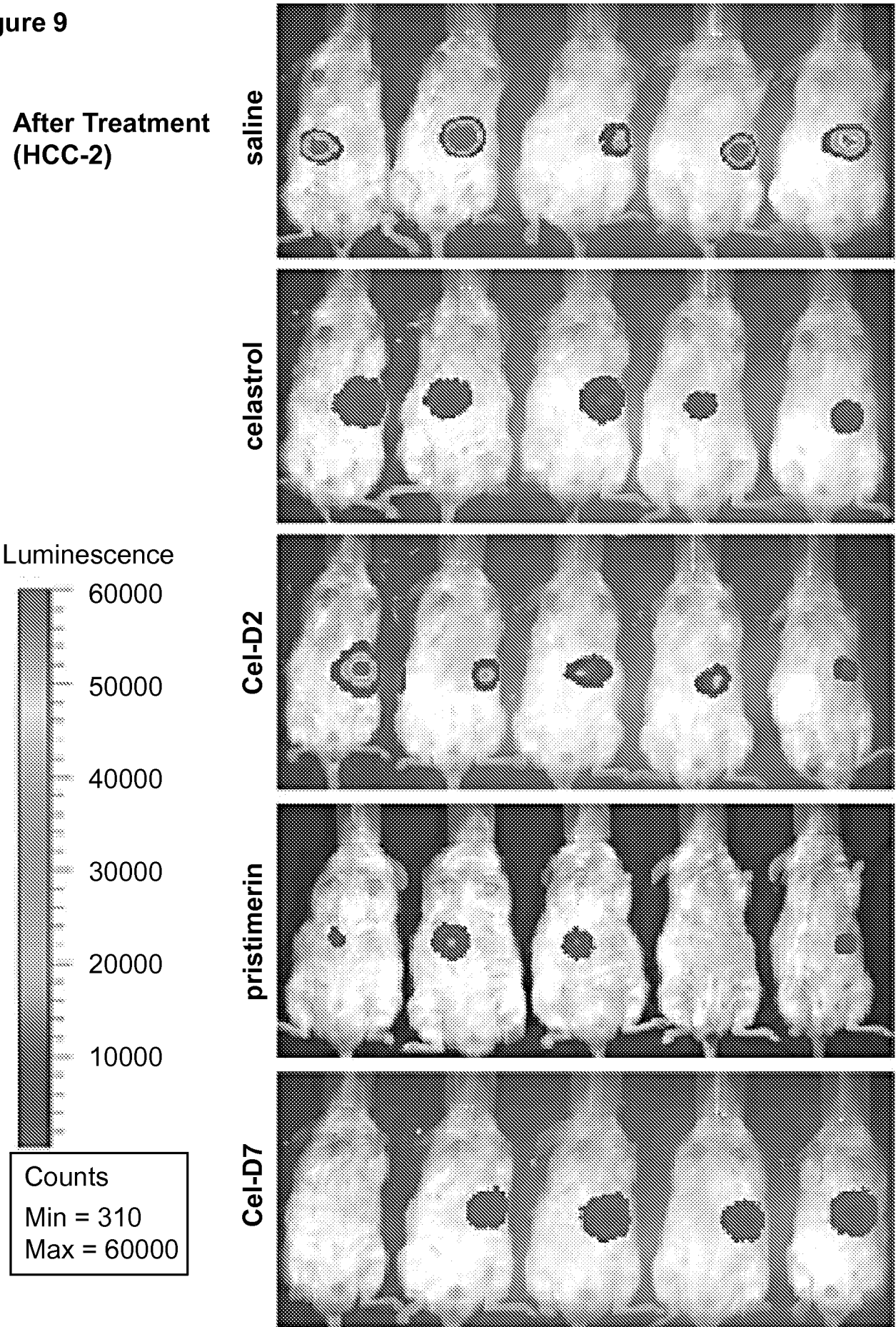


Figure 9

D

After Treatment
(HCC-2)



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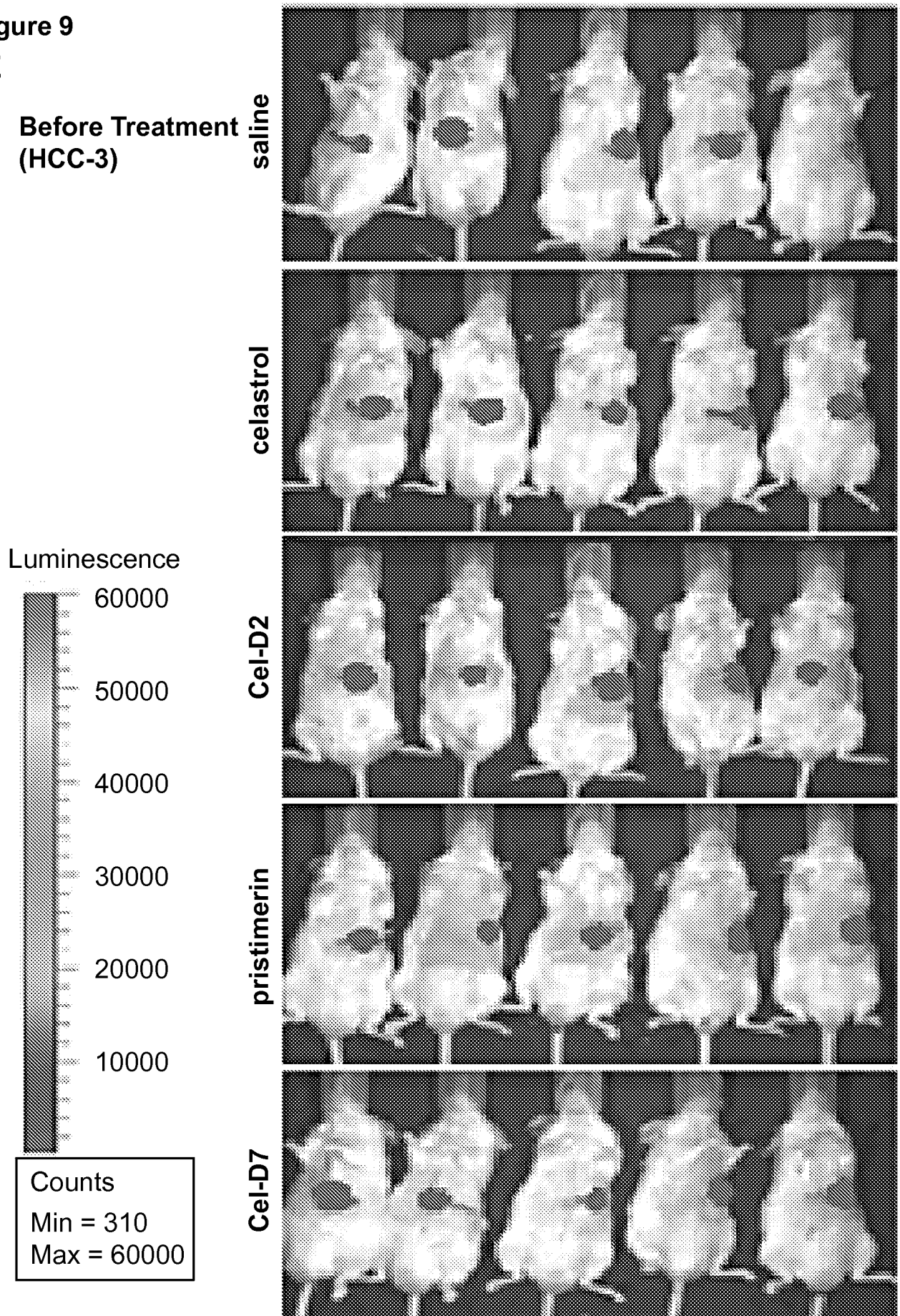
Figure 9**E****Before Treatment
(HCC-3)**

Figure 9
F
After Treatment
(HCC-3)

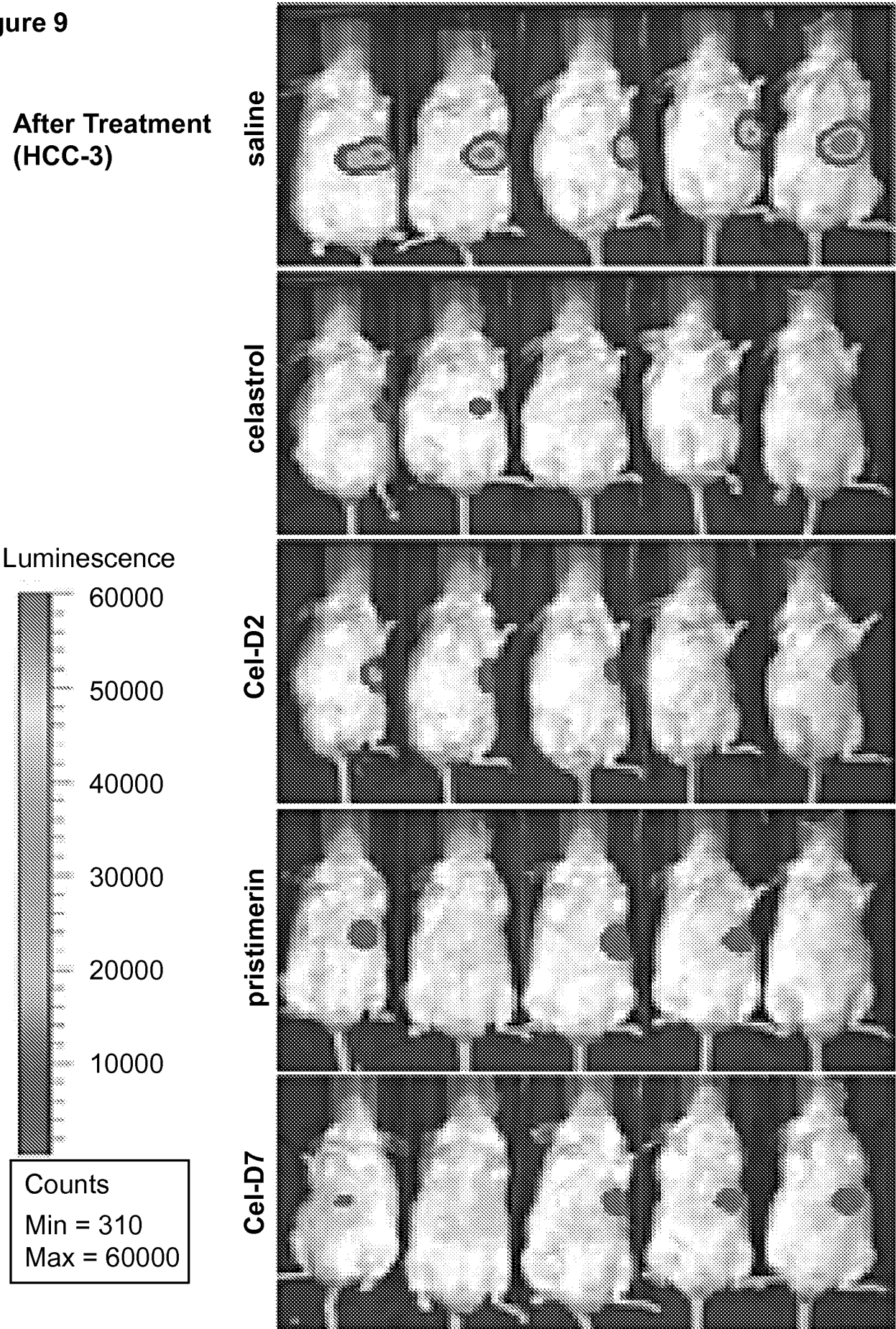
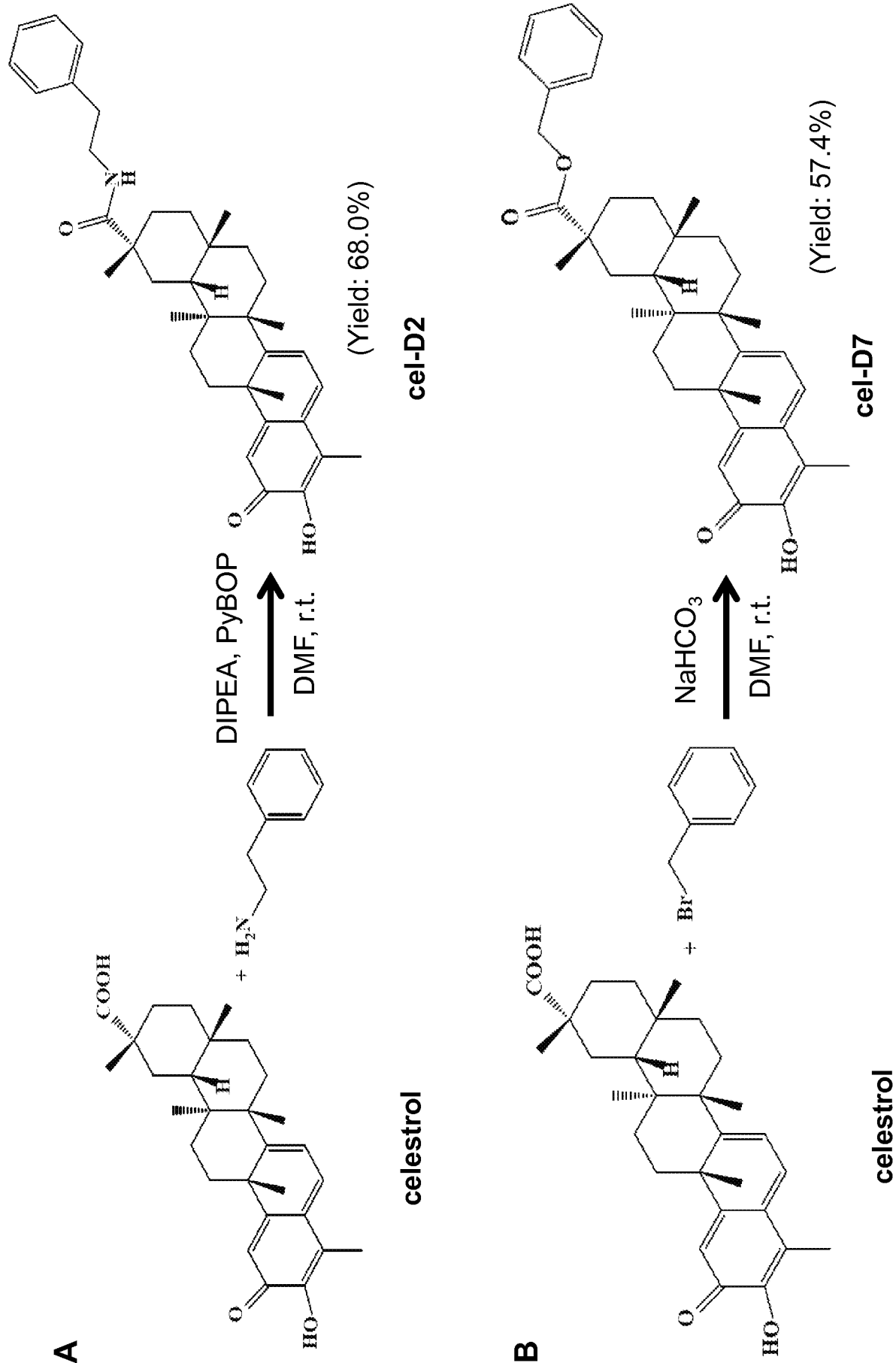
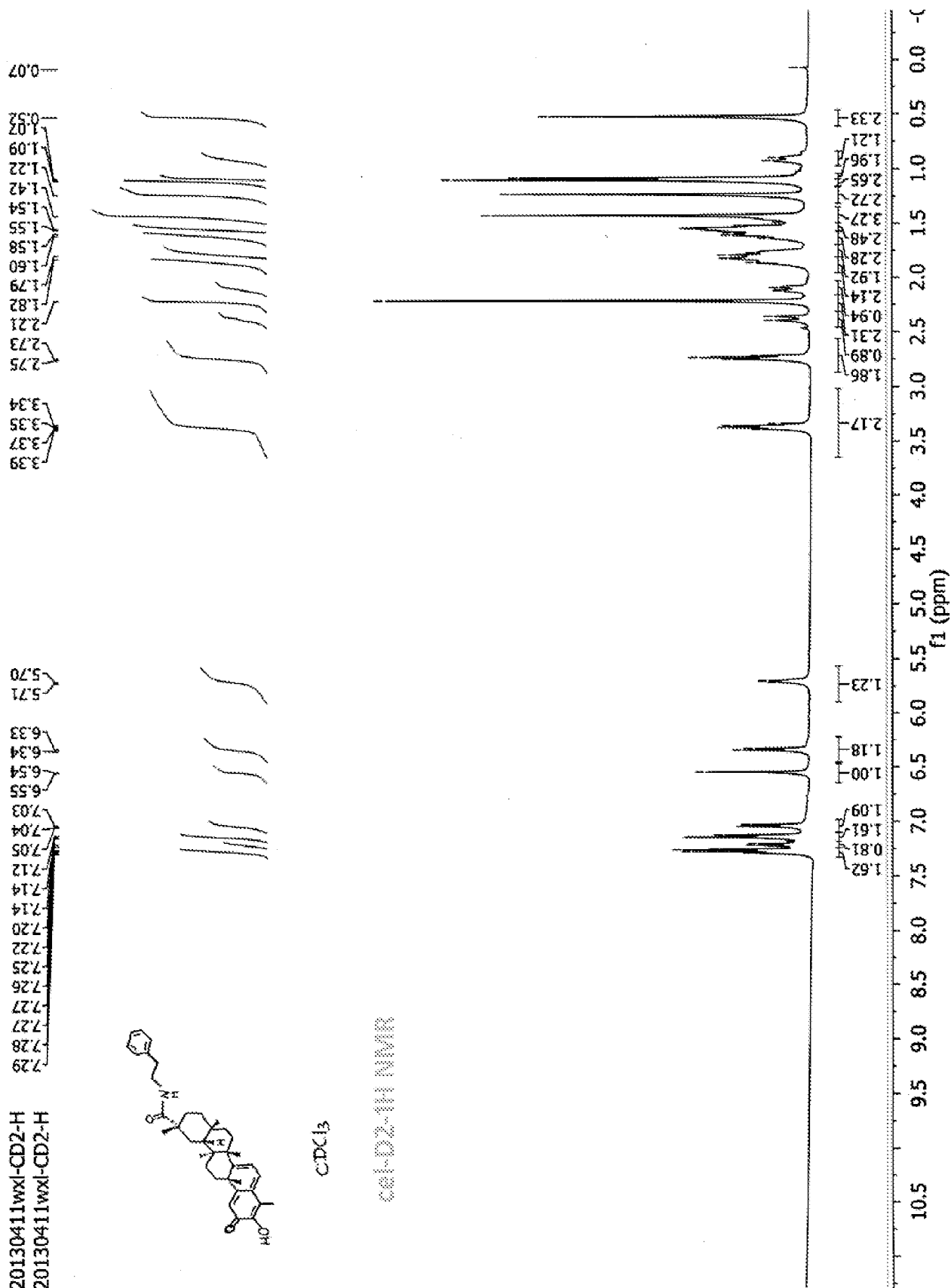


Figure 10



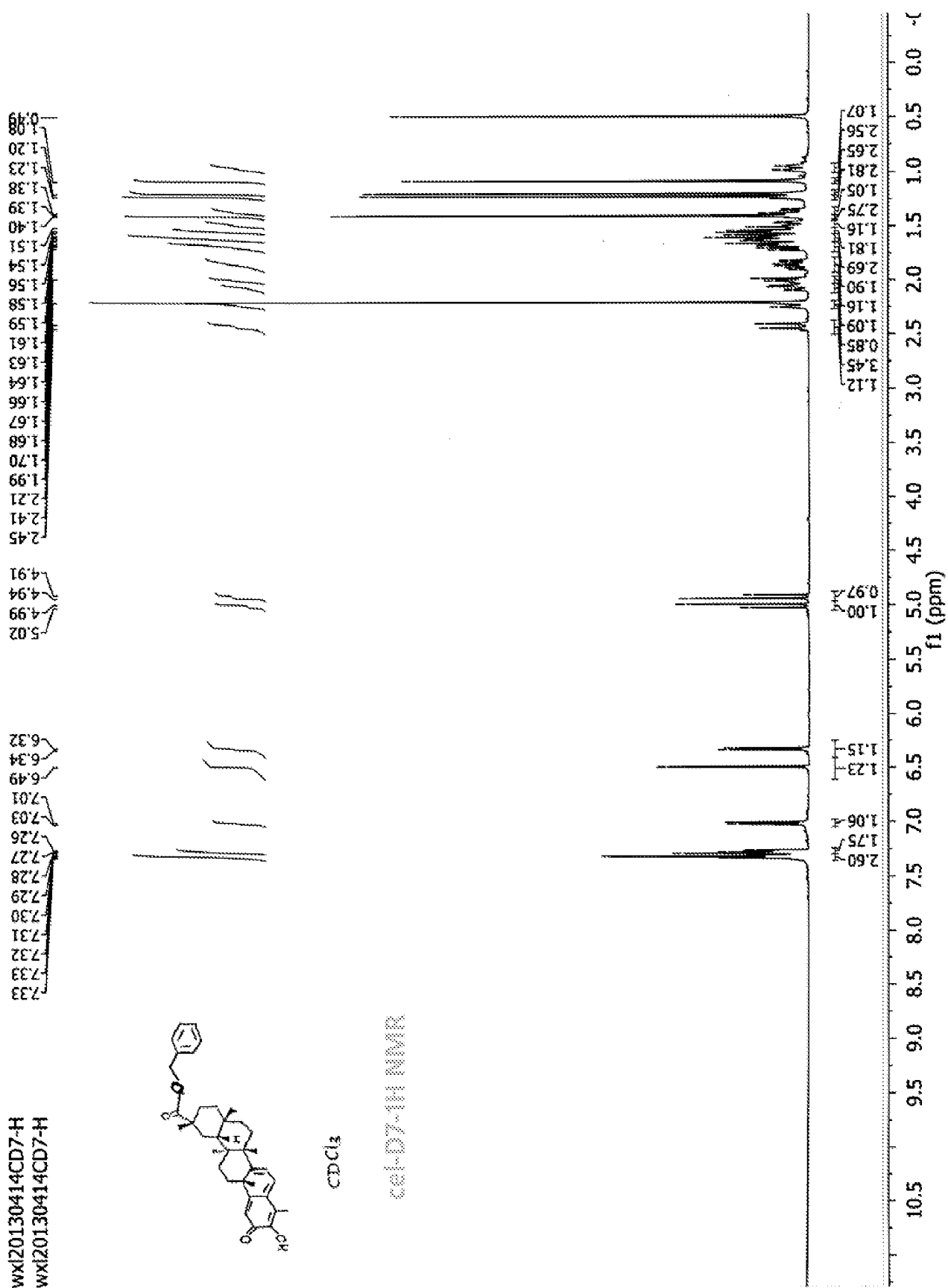
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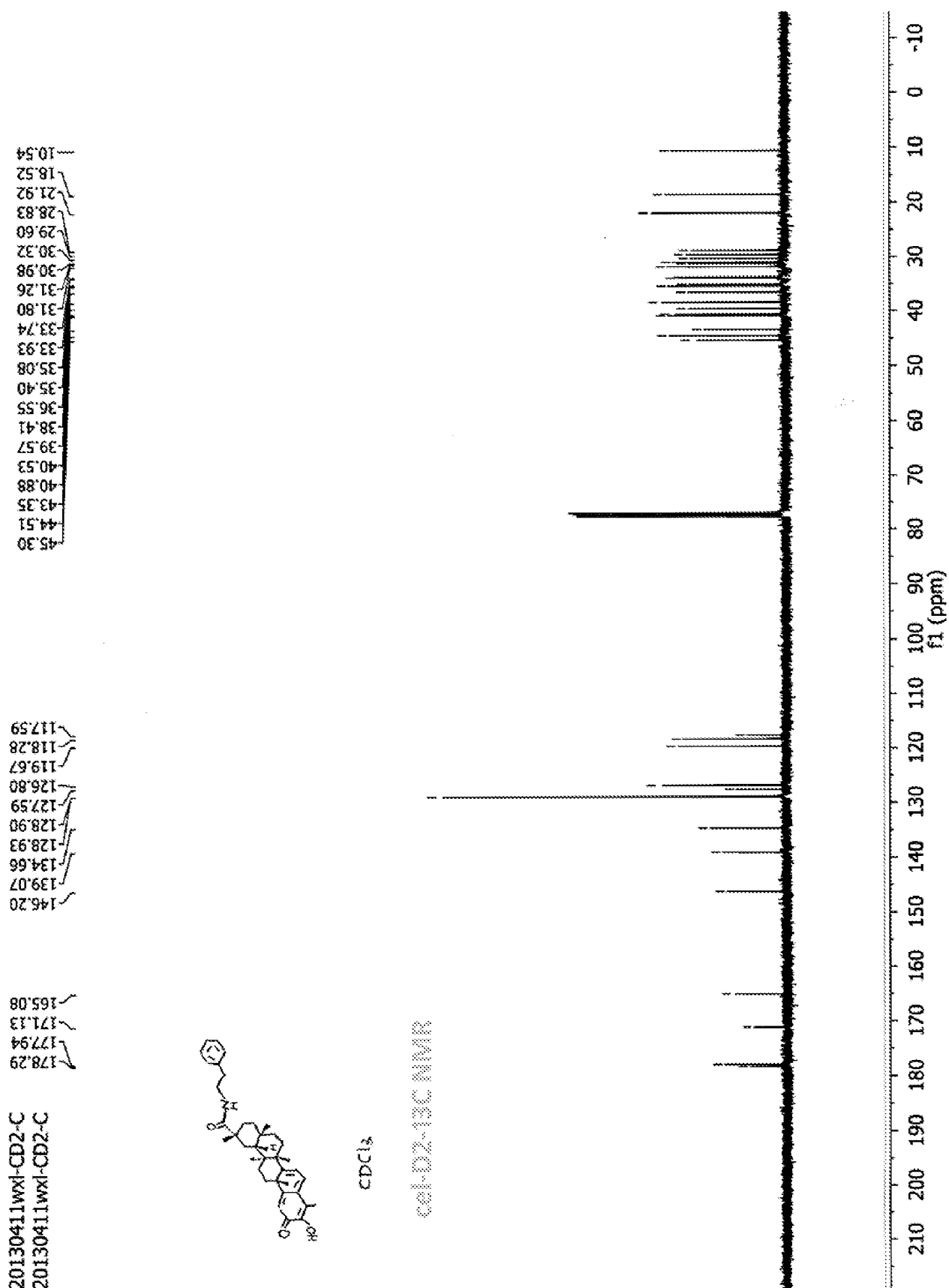
Figure 11



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Figure 12





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Figure 14

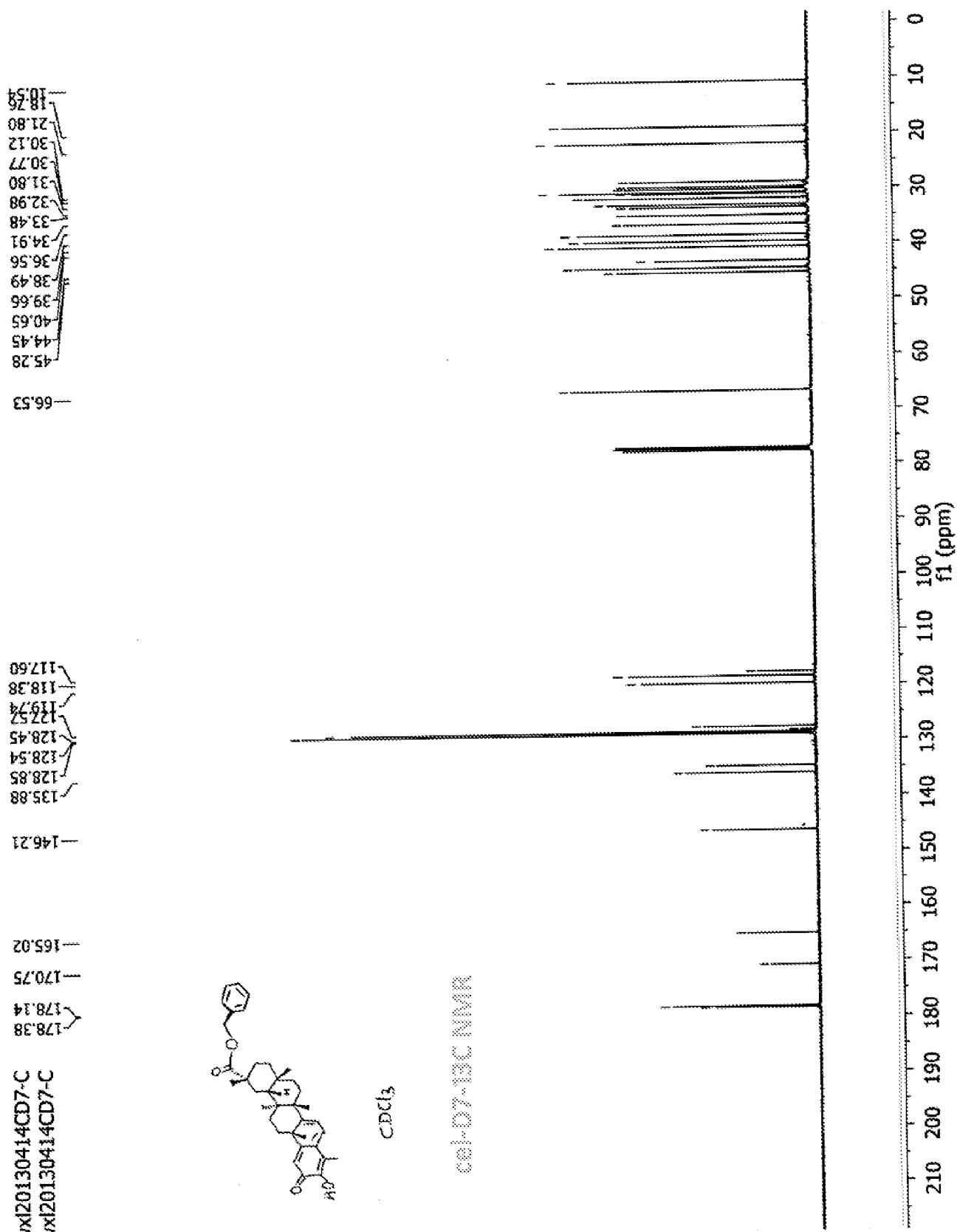


Figure 15

Mass Spectrum SmartFormula Report

Analysis Info
 Analysis Name D:\Data\HRAM\130415\data\WangX_41769_CD2_02_92_01_6857.d Acquisition Date 4/15/2013 2:10:28 PM
 Method loopin_pos_50_1200.m Operator BDAL@DE
 Sample Name WangX_41769_CD2_02 Instrument / Ser# microTOF-Q II 10292
 Comment

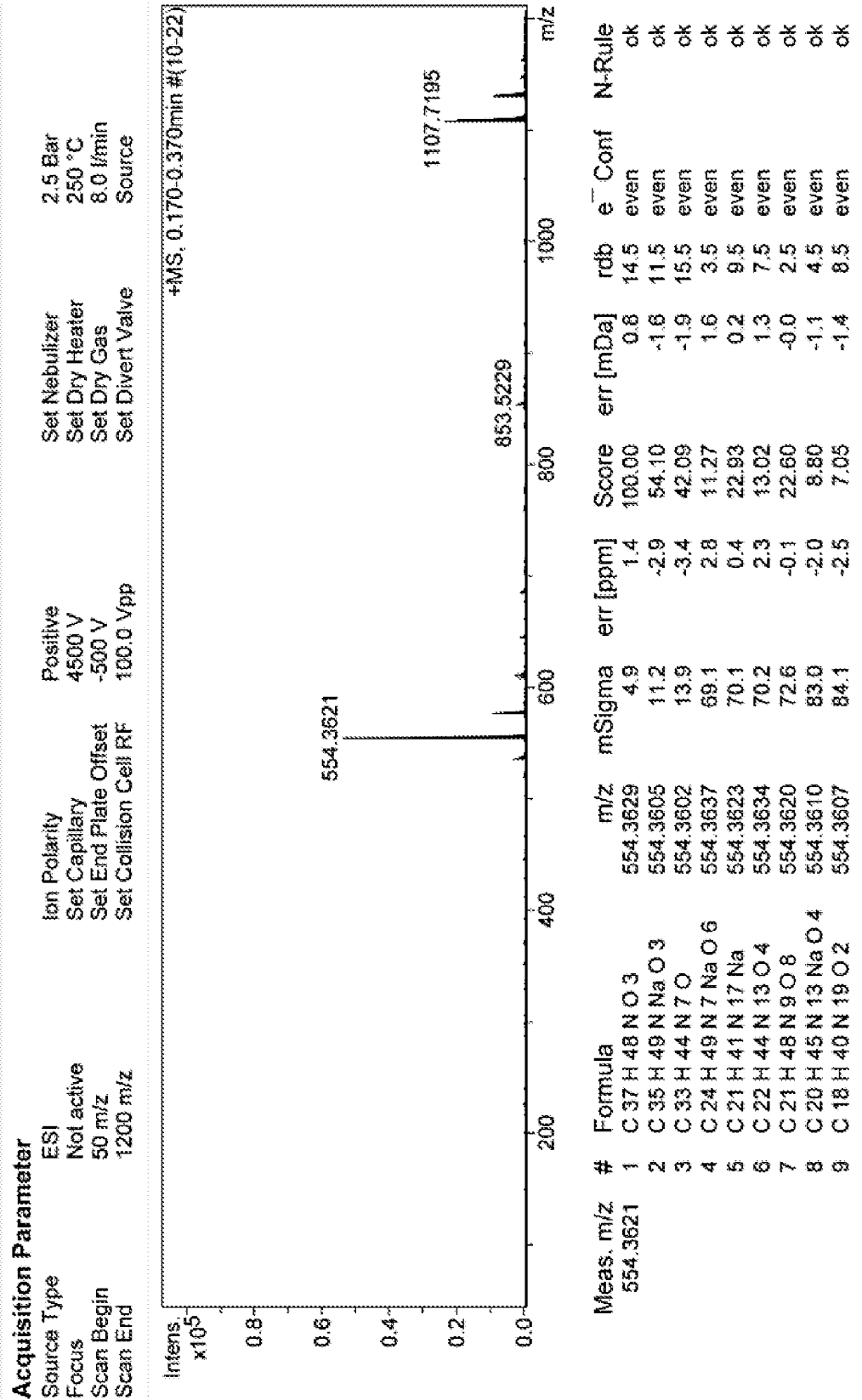


Figure 16

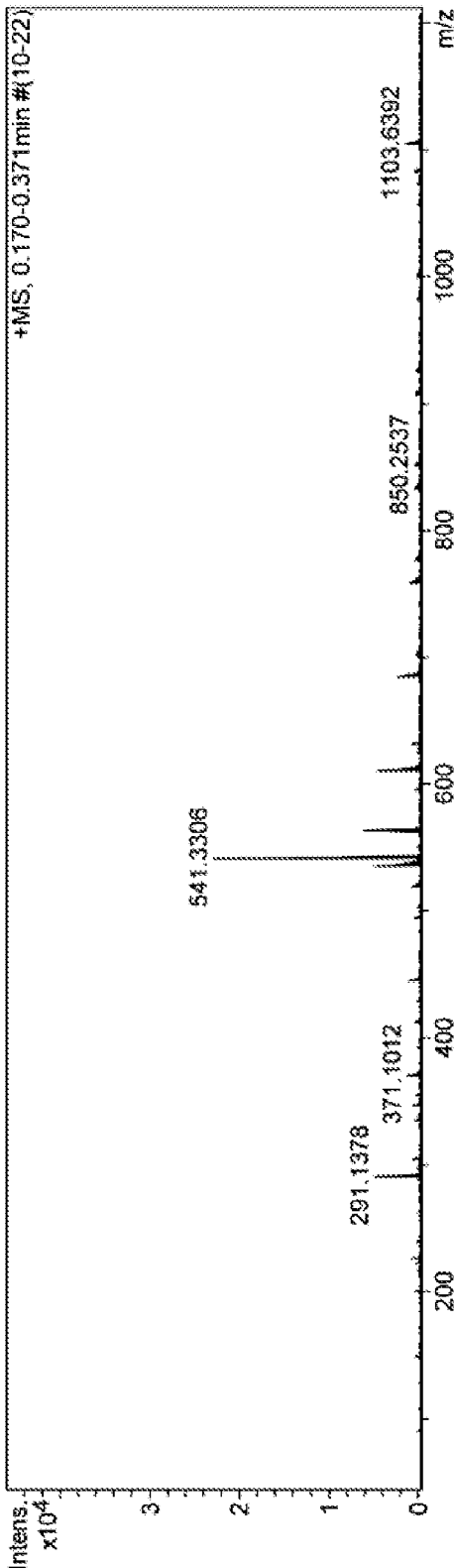
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name D:\Data\HRAM\130415\data\WangX_41769_CD7_02_93_01_6858.d Acquisition Date 4/15/2013 2:14:25 PM
Method loopin_pos_50_1200.m Operator BDAL@DE
Sample Name WangX_41769_CD7_02 Instrument / Ser# microTOF-Q II 10292
Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	2.5 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	250 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1200 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	mSigma	err [ppm]	Score	err [mDa]	rdB	e ⁻ Conf	N-Rule
541.3306	1	C ₃₆ H ₄₅ O ₄	541.3312	5.9	1.2	98.61	0.6	14.5	even	ok
	2	C ₃₅ H ₄₂ N ₄ Na	541.3302	10.0	-0.8	100.00	-0.4	16.5	even	ok
	3	C ₃₄ H ₄₅ NaO ₄	541.3288	11.1	-3.3	44.36	-1.8	11.5	even	ok
	4	C ₃₂ H ₄₁ N ₆ O ₂	541.3286	12.9	-3.8	34.73	-2.1	15.5	even	ok
	5	C ₃₇ H ₄₁ N ₄	541.3326	16.9	3.6	34.66	2.0	19.5	even	ok
	6	C ₂₃ H ₄₆ N ₆ NaO ₇	541.3320	59.3	2.6	16.37	1.4	3.5	even	ok
	7	C ₂₁ H ₄₁ N ₁₂ O ₅	541.3317	69.2	2.1	13.74	1.1	7.5	even	ok
	8	C ₂₀ H ₃₈ N ₁₆ NaO	541.3307	69.8	0.1	22.92	0.1	9.5	even	ok
	9	C ₂₀ H ₄₅ N ₈ O ₉	541.3304	71.6	-0.4	20.12	-0.2	2.5	even	ok
	10	C ₁₉ H ₄₂ N ₁₂ NaO ₅	541.3293	82.0	-2.4	7.62	-1.3	4.5	even	ok
	11	C ₁₇ H ₃₇ N ₁₈ O ₃	541.3291	83.1	-2.9	6.05	-1.6	8.5	even	ok

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/39415

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07J 53/00 (2015.01)

CPC - C07J 75/00; C07J 53/00; C07J 17/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C07J 53/00 (2015.01)

CPC: C07J 75/00; C07J 53/00; C07J 17/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 540/47, 548/528, 544/154, 544/380

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PATBase; Keyword limited: Celastrol; celastrol derivative/analog/analogue/amide/ester; tetradecahydronicene-2-carboxamide; hsp90/cdc37; carcinoma; hepatocellular carcinoma; celastrol carcinoma; celastrol hepatocellular carcinoma; celastrol cancer; heat shock protein; heat shock protein celastrol; chaperone celastrol

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2009/0054438 A1 (RONAI, ZA et al.) 26 February 2009 (26.02.2009), entire document, especially: para [0024]; para [0048], compound III; para [0049]; para[0050]; para [0051]; para[0071]; para [0084].	1,2,14-16, 21-23 ----- 3-13, 17-20
Y	ZHANG, T et al. "Characterization of Celastrol to Inhibit Hsp90 and Cdc37 Interaction", Journal of Biological Chemistry. 2009. Vol 284 (51), pp 35381-35388, entire document, especially: abstract; pg 35381, col 1, para 1; pg 35382, col 1, para 1.	3-13, 19
Y	US 5,650,167 A (ALLISON, AC) 22 July 1997 (22.07.1997), entire document, especially: col 1, ln 54-65; col 2, ln 31-40.	8,12-13,17-20
A	US 2011/0263693 A1 (VINSON-HIERONYMUS, H et al.) 27 October 2011 (27.10.2011), entire document.	1-23

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 September 2015 (11.09.2015)

Date of mailing of the international search report

07 OCT 2015

Name and mailing address of the ISA/US

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